THE RELATION BETWEEN THE BASAL METABOLISM AND THE ENDOGENOUS NITROGEN METABOLISM, WITH PARTICULAR REFERENCE TO THE ESTIMATION OF THE MAINTENANCE REQUIREMENT OF PROTEIN [1]

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The energy metabolism of an animal may be reduced to a minimum level by removing the gross influences tending to stimulate heat production, particularly muscular activity, the influence of food, and the stimulus of cold. This basal metabolism represents for each individual the minimum rate of energy expenditure compatible with the maintenance of life. It measures, therefore, the maintenance requirement of energy of the animal, a quota of energy that must be provided by the food consumed if the normal life activities are to be maintained.

Similarly, the nitrogen metabolism may be reduced to a minimum level by removing certain gross influences that tend to stimulate the wastage of nitrogen from the body. In this case, the disturbing influences, while few in number, are somewhat more difficult to remove. Muscular activity is not among these influences, since it does not appreciably affect nitrogen wastage from the body, except as it may induce a negative

[1] The experimental data reported in this article have been taken from the thesis of D. B. Smuts submitted in 1932 to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Animal Husbandry. This paper was prepared for publication by H. H. Mitchell.

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balance. There are only two requirements for the attainment of a minimum level of nitrogen output, the 'endogenous metabolism' of Folin. The first is that the protein intake should be reduced preferably to zero, or at least to such a level, depending upon the character of the dietary protein, that it will exert no appreciable influence on the rate of nitrogen excretion by the kidneys. The second is that the energy intake should be great enough to protect the nitrogenous constituents of the tissues from catabolism in so far as this is possible. Under these conditions, as Folin ('05) first discovered, the nitrogen output in the urine is reduced to a very low level, which may be considered to measure in a very true sense the requirement of nitrogen (protein) by the animal for the maintenance of life,[3] specifically for the maintenance of the nitrogenous integrity of the tissues (Mitchell, '26, '29; McClellan and Hannon, '32). Folin further showed that, on a creatine-free diet, the creatinine output in the urine is unaffected by the protein intake, and hence presumably that creatinine is a product only of the endogenous nitrogen metabolism.

It seems reasonable to believe that there is a relationship, directly or indirectly causal in nature, between the minimal catabolism of the nitrogenous constituents of protoplasm and the minimum rate at which energy is liberated, as measured

[3] This interpretation of the 'endogenous metabolism' is quite in accordance with the original conception of Folin. In this paper, as in the early studies of McCollum and Steenbock ('12) on the pig, the endogenous level of protein metabolism is taken as that level reached on a nitrogen-free diet when consumed in amounts to cover the energy requirements of the animal. If this criterion of the attainment of the endogenous protein metabolism differs at all from that of Folin, it is in the inclusion of a certain amount of protein metabolism of the exogenous type, but the inclusion of such a fraction seems inherently improbable, since with the rat this level of nitrogen excretion is not depressed by an increase in the consumption of nitrogen-free food, if the intake of food energy is slightly above the requirement of maintenance (Mitchell, '24). That nitrogen equilibrium may be attained on an intake of digestible nitrogen—this term being used in the ordinary sense—just sufficient to cover the endogenous losses in the urine, has been shown by McCollum and Steenbock ('12, p. 61) for the pig, using corn or oat protein, and by Mitchell ('24, p. 889) for the rat, using the mixed proteins of milk and lactalbumin.

by the basal heat production. Both represent the 'idling speed' of the organism, one with reference to the catabolism of a particular class of cellular constituents characterized by their content of nitrogen, the other with reference to the total catabolism of the organism. The total energy liberation of the 'idling' organism includes the energy liberated in those chemical reactions constituting the endogenous catabolism and yielding ultimately the end-products of this catabolism. From this fact only one would expect a measurable correlation between the two, the size of which would depend upon the fraction of the total energy transformations represented in the energy liberated by these endogenous reactions involving the nitrogenous constituents of the tissues.

Only a few investigations have been concerned directly with the relation existing between the basal metabolism and the endogenous nitrogen metabolism. In 1914, Palmer, Means and Gamble studied the relation between basal metabolism and creatinine elimination, with the expressed hope of finding a better basis for the comparison (and prediction) of the basal metabolism of different individuals than that afforded by body weight or body surface. For eight men subjects the average number of calories per milligram of creatinine excreted was 0.98, and for nine women subjects it was 1.26. Although the men varied in weight from 58 to 94 kg., the individual ratios were remarkably constant, possessing a coefficient of variation of only 4.7. The women subjects varied from 48 to 80 kg. in weight and their ratios varied to the extent of 5.2 per cent.

If one may assume that the creatinine elimination is a function of the protein content of the body, then the work of Palmer, Means and Gamble receives confirmation from the investigations of Kohlrausch ('29–'30) concerned with methods for the determination in vivo of the chemical constituents of the body. Kohlrausch experimented with four dogs, varying in weight from 3.8 to 11.5 kg., some of which had been trained to develop their muscular tissue, while others were untrained. By determination of the basal metabolism and actual analysis

of their carcasses, he found a constant relationship between the former and the protein content, such that the basal heat per minute (in calories) divided by the two-thirds power of the weight of protein in grams equaled 2.30.

Terroine and Garot ('26) applied to eight species of animals (rat, guinea pig, rabbit, dog, sheep, man, horse and cow) the same type of investigation as that of Palmer, Means and Gamble. They found, however, that the ratio of creatinine elimination (in milligrams) to basal heat (in calories $\times$ 100) was not constant, but varied from 29 for the rat to 135 for the horse. Hence, while the ratio may be constant for any one species, it is apparently not constant between species.

The most direct attempt that has heretofore been made to establish a relationship between the basal heat production and the output of endogenous nitrogen has been made in the laboratory of Terroine at Strassburg. Terroine designates the sum of the urinary and fecal nitrogen on a nitrogen-free diet as the total endogenous nitrogen. His basal heat determinations were made by the Haldane gravimetric method (1892), and generally extended over several hours, during which minimal activity, but not complete repose, prevailed. In the large majority of cases, if not in all, the two determinations were not made upon the same animal, the basal heat generally being computed from the numerous determinations reported from previously published experimental data.

In the first paper of this series, Terroine and Sorg-Matter ('27) reported average ratios on three mice, six rats, three pigeons, three chickens and three rabbits, and computed ratios for man and the dog. These specific ratios ranged from 2.30 to 2.90 mg. of endogenous nitrogen per calorie of basal heat, averaging 2.61 mg. Their coefficient of variation is 9.4.

In a second paper by the same authors ('28), the ratio of endogenous nitrogen to basal heat was studied with a number of mice and pigeons exposed to different environmental temperatures, some below the critical temperature and some above. It was found at the lower temperatures that the elimination of endogenous nitrogen increased as well as the

basal metabolism and somewhat in the same proportion, so that the ratio of the two was not greatly disturbed. However, in these experiments the ratios were much lower than in the first experiment, averaging 2.18 to 1, the range being from 1.97 to 2.45. No food intakes are reported, so that one is unable to determine whether the observed increases in the total nitrogen excretion were associated with adequate food intakes, or whether they included an increment in nitrogen metabolism induced by an inadequate intake of food energy.

In a third paper by Sorg-Matter ('28), three rats and nine roosters of varying weights and ages were studied. Again the endogenous nitrogen per calorie of basal heat fell within a small range, 2.20 to 2.53 mg., averaging 2.37 mg. Apparently, the more intense basal metabolism of the young animal is associated with a more intense endogenous nitrogen metabolism. The same sort of study on two pigs at widely different body weights was later reported by Terroine, Boy, Champagne and Mourot ('33). The endogenous nitrogen elimination of the pigs (in urine only) was measured at weights of 13 to 14 kg. and again at a weight of 132 kg. The weights of urinary nitrogen per calorie of basal heat varied from 1.8 to 2.0 mg. The fecal nitrogen was determined only at the heavier weights, and at these weights the total endogenous excretions (urine and feces) per calorie of basal heat were 2.3 and 2.1 mg. Summarizing all of their work in this article, it is concluded that the "law of the constant relationship of minimal nitrogen and energy output" holds for the whole series of homeotherms from mouse to pig, with body weights ranging from 1 to 8800 and heat production per kilogram of body weight ranging from 1 to 40.

Ashworth and Brody ('33 a and '33 b) have also published the results of some studies on the relation existing between the basal energy metabolism of rats and their minimum excretion of endogenous nitrogen. With different rats they report, contrary to Terroine, a very variable ratio, ranging from about 0.7 to 2.0 mg. of endogenous nitrogen per calorie of basal heat, and averaging about 1.4 mg. No effect of age

on this ratio is evident in their work, either from a group of rats upon which both determinations were made, or from another group for which the basal metabolism was estimated.

The subject of Terroine's investigations is one of scientific importance, since it attempts to associate causally two basic phases of animal metabolism hitherto considered separate and distinct. Incidentally it confirms strikingly Folin's conception of the endogenous nitrogen metabolism, the constancy, or even the reality, of which has been questioned by some investigators. Furthermore, the subject possesses practical significance. The basal metabolism of animals measures their basal (or maintenance) requirement for food energy, while the endogenous loss of nitrogen measures their basal (or maintenance) requirement for nitrogen. The basal metabolism can be measured satisfactorily if the proper equipment is available, but the endogenous nitrogen elimination is difficult to measure, except for small laboratory animals and for pigs, because of the difficulties involved in compounding and feeding suitable rations. If there is a constant relation between the two, it should be possible to estimate the endogenous nitrogen (and the maintenance requirement of protein) of any animal from its basal metabolism, as measured, or as estimated, from body size.

However, Terroine's data are not completely satisfactory, for the following reasons:

1. The inclusion of fecal nitrogen with urinary nitrogen combines two factors of different antecedents. The urinary nitrogen on a nitrogen-free diet is related to body size, and, above a certain minimum intake, is not related to food intake (Mitchell, '24). The metabolic nitrogen of the feces, however, though of body origin, is mostly related to the processes of digestion and varies directly with the amount of digestive work done, as measured roughly by the dry matter consumed. Only a small fraction of the metabolic nitrogen of the feces of rats is unrelated to the digestion on normal intake of food (Schneider, '34). Hence, the total endogenous nitrogen of Terroine, including both fecal and urinary nitrogen, will vary

in size directly with the intake of food, although the true endogenous nitrogen, in the sense of Folin, is not so affected.

2. Terroine, in practically all of his work, determined the endogenous nitrogen on each experimental animal, but estimated the basal metabolism from average values obtained on other animals of the same species and in some cases in other laboratories. However, individual differences, both with regard to basal metabolism and to endogenous nitrogen, have been observed and are undoubtedly factual. The use of an observed value for one factor and an estimated value for the other will obscure the relationship that may exist between the two, so that the ratios obtained will exhibit a fictitious variability.

3. The basal metabolism determinations of Terroine and Trautmann, which were used in many of the calculations, were not made under conditions of absolute repose of the experimental animals. They are, therefore, to some extent too high and too variable.

4. The observed ratios of endogenous nitrogen to basal calories, while suggestive of a constant relationship, exhibit considerable variation, particularly as between different sets of data reported in different publications. The data of Ashworth and Brody are even more at variance with the conception of a constant relationship.

The following investigations were inspired by the work of Terroine and colleagues, but were carried out with the above criticisms in mind. It was hoped, by correcting what appeared to be defects in the experimental procedures and technic of Terroine, to secure more convincing evidence of a constant relationship between the endogenous nitrogen elimination of animals of different species and their basal metabolism. Such evidence has, the writer believes, been secured.

PLAN OF EXPERIMENT

The experiments were carried out upon five species of animals, the mouse, rat, guinea pig, rabbit and pig. The mice ranged in weight from 20 to 30 gm., averaging 24, and included two females and seven males. Of the twenty-three rats

in the experiment, fifteen were males and eight females. The former averaged 243 gm. in weight (202 to 318), and the latter 186 gm. (170 to 227). Of the nine guinea pigs, six were males; the body weights averaged 414 gm. (300 to 489). The ten rabbits were all males and ranged in weight from 1440 to 2740 gm., averaging 2072 gm. The five pigs also were all of the male sex; their weights were 62.3, 66.7, 68.0, 69.5 and 71.7 kg. Upon each of these fifty-six animals the endogenous nitrogen was determined and at least one basal metabolism measurement was made. To permit expression of the latter measurement in the usual terms of calories per square meter of body surface per day, it was necessary to determine the surface areas of a number of mice, guinea pigs and rabbits, for which species good prediction formulas did not seem to be available, and to devise mathematical equations relating surface area to body weight. The results of this phase of the study have been published elsewhere (Smuts, '33).

The general plan of procedure was, first, to place each animal for at least 7 days upon a low-protein (10 per cent), but otherwise complete diet (table 3). The animal was then fasted for a period of time, varying with the species, until the post-absorptive condition was established. For mice, guinea pigs, rabbits and pigs it was considered necessary, in auxiliary tests, to determine the period of fasting required for this purpose. The basal metabolic rate was then determined.

The animal was next placed upon a low-nitrogen diet until its nitrogen metabolism was reduced to the endogenous level. The length of time required in the various species of animals for the depletion of the so-called 'deposit protein' was studied, and, at the termination of this period, the urine was collected for 7 days and its nitrogen content determined.

Those animals upon which a second basal metabolism determination was to be made were then, after the period of low-nitrogen feeding, put again upon the complete standard diet for 7 days and then fasted as before.

METHODS

Basal metabolism. The basal metabolism of the mice, rats, guinea pigs and rabbits was determined by a modification of the gravimetric method first proposed by Haldane (1892). The respiratory quotient was determined by the ordinary technic in a period of 6 to 8 hours. During this time the animals, although confined in a small chamber, exhibited more or less activity, which is incompatible with a basal level of metabolism. Hence the basal production of CO_2 was determined during short periods (ranging in toto from 40 to 60 minutes) of complete muscular repose. During these periods, the animal was under constant observation, and at the initiation of any appreciable activity the period was interrupted, to be resumed again only when the animal had become quiet and had stayed quiet for at least 5 minutes. The carbon dioxide produced during these truly basal periods was collected in a separate chain of absorption bottles, to which the ventilating current could be diverted by means of a three-way stopcock.

The basal metabolism of the experimental pigs was determined in a pneumatic chamber.[4] The chamber has a volume of 1190 liters. During a test, the air within the chamber was vigorously stirred by an electric fan and its moisture content was kept down by exposure to sticks of P_2O_5 contained in a shallow porcelain dish. Samples of air were taken from the chamber at the beginning and the end of a test and analyzed for CO_2 and O_2. The gaseous exchange of the animal was computed from, a) the difference in composition of the chamber air at standard temperature and pressure; b) the air volume of the chamber, and, c) the elapsed time. The tests generally continued for about 60 minutes, during which time the CO_2 content of the air reached a concentration of about 1 per cent. To prevent any appreciable leakage of air into or out of the chamber, the temperature within the chamber was

[4] Grateful acknowledgment is made to the Graduate School of the University of Illinois which provided funds for the construction of this pneumatic chamber. Without this timely aid, it would not have been possible to extend the investigation to pigs.

not allowed to rise above the temperature outside, a result secured by continuously running water over it. What small leakage occurred, was corrected for on the basis of the nitrogen content of the chamber air.

With pigs the influence of muscular activity upon the heat production could not be as easily removed as with the smaller animals. The only recourse was to discard the measurements obtained during periods of appreciable activity, which was possible, because the pig could be observed through a window in the chamber. The test was then repeated until a period of absolute rest was obtained.

Both methods of determining the basal metabolism neglect protein metabolism, since the total respiratory quotients are used in the calculations as non-protein respiratory quotients. The error introduced by this simplification, which is commonly made in such work, is negligible under basal, and even under most conditions, rarely exceeding 2 per cent.

The significance and reproducibility of a basal metabolism determination depend very largely upon the removal of the stimulus to heat production exerted by the last meal. The experimental rats were fasted for at least 17 hours before the basal measurements were made. On the basis of the work of Benedict and MacLeod ('29 a), it was concluded that post-absorptive conditions prevailed after this time. For the other species of animals, special tests were made to determine the time of removal of the food stimulus.

A study was undertaken with five mice to determine the necessary fasting period, from which it appears that 10 hours are sufficient for the establishment of a constant rate of heat production. After 10 hours of fasting, the R.Q.'s were usually in the neighborhood of 0.72 to 0.73, which constitutes additional evidence that the mouse at this time is catabolizing its own tissues.

A similar investigation with four guinea pigs showed that, under the conditions of feeding imposed, at least 20 to 22 hours of fasting were necessary for the attainment of a true basal condition of metabolism.

The results obtained with three rabbits are interesting. The heat production was determined at twelve hour intervals for periods of 72 or 84 hours, with the results given in table 1. The low rates of heat production for all three rabbits after 24 hours of fast are difficult to understand. A constant level of heat production was obtained only after 60 hours of fast.

TABLE 1

The heat production of rabbits after different periods of fasting

ANIMAL NO.	BODY WEIGHT	TIME FASTED	DAILY HEAT PRODUCTION	HEAT PRODUCTION PER DAY PER M^2 OF BODY SURFACE	S.Q.
	gm.	*hr.*	*cal.*	*cal.*	
R1	2330	10	206.9	1250	0.88
	2250	24	106.8	655	0.85
	2310	24	105.7	641	0.86
	2240	36	126.6	777	0.83
	2215	36	121.0	747	0.81
	2240	48	112.0	688	0.77
	2170	60	94.2	587	0.74
	2240	72	90.5	556	0.71
	2155	84	102.1	638	0.70
R2	2308	10	198.4	1204	0.90
	2200	24	116.5	722	0.81
	2120	36	152.8	984	0.79
	2100	48	147.4	931	0.79
	2120	60	119.6	755	0.73
	2110	72	118.3	747	0.70
R3	2550	24	125.7	730	0.81
	2500	42	159.0	932	0.77
	2570	66	120.5	698	0.75
	2565	72	122.9	712	0.69

The rate of decline in heat production during fast for two pigs is shown by the values contained in table 2. The decline is very gradual, in spite of the simple structure of the gastro-intestinal tract in the pig. Only after 96 hours of fast may the heat production be considered to have reached a fairly constant level. This finding is in agreement with the work of Lenkeit ('31), who reported that only after 4 days had the major portion of a test meal completely traversed the

alimentary canal of the pig. Capstick and Wood ('22–'23) found that the metabolism of a pig decreased after feeding for about 90 hours.

Hence, in the routine measurement of the basal metabolism of the animals used in this experiment, the following periods of fast were imposed: mice 10 hours, rats 17 hours, guinea pigs 22 hours, rabbits 60 hours and pigs 96 hours.

To remove the stimulus of cold, which is also incompatible with a basal condition of metabolism, it is necessary to maintain an environmental temperature above a certain minimum, which is known as the 'critical' temperature. The following

TABLE 2

The heat production of pigs after different periods of fasting

TIME FASTED	PIG NO. 4		PIG NO. 5	
	R.Q.	Heat production per m^2 per hour	R.Q.	Heat production per m^2 per hour
hr.		*cal.*		*cal.*
4	0.99	74.5	1.20	86.9
24	1.00	85.0	0.83	63.9
48	0.85	51.0	0.79	52.6
72	0.80	51.5	0.78	39.0
96	0.79	45.2	0.80	38.7
120	0.75	42.4	0.78	35.3
144	0.74	44.2	0.79	32.0

minimal temperatures were observed throughout this work; the evidence, where available, that these temperatures are critical will be found in the literature articles cited:

Mouse, 29 to 30°C. (Aszódi, '21; Benedict and Fox, '32–33).
Rat, 28°C. (Benedict and McLeod, '29 b).
Guinea pig, 32 to 33°C. (Murschhauser, '12; Rubner, '02).
Rabbit, 27 to 28°C.
Pig, 21°C. (Capstick and Wood, '22; Deighton, '29).

It was assumed that the rabbit, with its ample fur covering, would not exhibit a cold stimulus at a temperature as high as 27 to 28°, though no experimental evidence on this point was obtained from the literature.

The endogenous nitrogen excretion. The low-nitrogen rations used in reducing the experimental animals to the minimum endogenous catabolism are described in table 3. It will be noted that the ration used for pigs contained no considerable source of nitrogen, with the exception of 1 per cent of

TABLE 3

Percentage composition of the experimental rations

INGREDIENTS	STANDARD RATION	NITROGEN-LOW RATIONS FOR				
		Rats	Guinea pigs	Mice	Rabbits	Pigs
Dried ether-extracted whole egg	....	5.02	5.02	5.02	5.02	....
Casein	12.14	....	....	....	....	....
Salts	4.00	4.00	4.00	4.00	4.50[1]	4.00[2]
Butterfat	10.00	10.00	12.00	10.00	....	....
Cod liver oil	....	....	....	....	2.00	1.00
Mazola oil	....	....	....	....	10.00	....
Sodium chloride	1.00	1.00	1.00	1.00	1.00	....
Sucrose	10.00	10.00	10.00	10.00	10.00	....
Cellu flour[3]	4.00	4.00	20.00	4.00	25.00	....
Cellomass	....	....	....	....	1.00	2.00
Yeast	....	....	....	....	1.00	1.00
Starch	58.86	65.98	47.98	65.98	40.48	92.00
Per cent nitrogen		0.65	0.67	0.65	0.68	0.14
Calories per gram[4]		4.0	4.2	4.0	4.3	4.0

[1] A modified Osborne and Mendel salt mixture, proposed by P. B. Hawk and B. L. Oser, 1931, Science, vol. 74, p. 369.

[2] Salt mixture for pigs. Steamed bone meal 15 parts, ground limestone 15, sodium chloride 15, magnesium carbonate 1.5, potassium carbonate 1.5, potassium sulphate 1.0, ferric chloride 0.75, potassium iodide 0.25.

[3] Cellu flour is a product put out by the Chicago Dietetic Supply House. By actual analysis it contains 37.8 per cent crude fiber, and only 0.015 per cent of nitrogen.

[4] Determined by the Parr oxygen bomb calorimeter.

yeast, while the rations used for the other animals contained approximately 5 per cent of dried ether-extracted whole egg. Mitchell and Carman ('26 c, p. 200) have shown that egg protein at this low level does not affect the output of either fecal or urinary nitrogen, while its presence in the diet maintains the appetite and the weight of experimental rats much

better than does a practically nitrogen-free diet. The cost of this egg preparation prevented its use in the pig experiment.

The period of time required to attain the endogenous nitrogen excretion will vary with the different species. It has been shown by Mitchell ('24, tables 15 and 16) and again by Mitchell and Carman ('26 c, table 17) that the rat rapidly adjusts itself to its endogenous nitrogen metabolism, only 3 or 4 days being required, and maintains a constant level, biologically speaking, for at least 10 days or 2 weeks. However, in the present investigation the experimental rats were kept for 6 days on the low nitrogen diet before the collection of urine was started.

To determine the necessary period of preliminary feeding for the other species of animals, tests were made upon three representatives of each species, the outcome of which is given in table 4. As a result of these tests, in all subsequent work the following periods of preliminary feeding were adopted: for mice, 5 days; for guinea pigs, 8 days; for rabbits, 15 days; and for pigs, 20 days.

The periods for the collection of urine were in all cases 7 days in length. The animals generally ate well during these tests, but if there was a distinct failure of appetite and a corresponding rapid loss in weight, the animal was discarded.

All of the animals were confined in individual dishes or cages, varying in size with the size of animal, but similar in design. In all cases, the animal rested upon a perforated floor, made of glazed earthenware (rats and mice), hardware cloth of ½ inch mesh (guinea pigs), aluminum alloy bars (rabbits), or heavy reinforced wire fencing of 1 inch mesh (pigs). The feces were readily passed through this floor, except in the case of the pigs, and were caught upon copper window screening suspended between the perforated floor and the bottom of the dish or cage to which the urine drained. In the metabolism receptacles in which the mice, rats and guinea pigs were confined, the urine was absorbed upon filter paper; in the rabbit and pig cages it drained along a sloping surface to a container below. The collections of urine were made daily

by the methods employed in this laboratory, the adequacy of which has been satisfactorily demonstrated. The completeness of the collections for rats and mice, about which some investigators are sceptical, was retested in connection with these studies, and percentage recoveries of 97.5 to 99.6 were obtained in nine trials.

TABLE 4

The adjustment of the urinary nitrogen to the endogenous level in mice, guinea pigs, rabbits and pigs. Results expressed in milligrams per day

DAYS ON LOW-NITROGEN RATION	MICE			GUINEA PIGS			RABBITS			PIGS		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
1	15.0	17.8	15.8									
2	14.6	17.4	15.0									
3	13.4	16.9	14.3	146								
4	13.0	16.9	14.5	144								
5	13.1	16.6	13.7	138	124	132						
6	13.3	17.8	14.6	128	123	122						
7		16.6		115	99	115	558			6292	3312	4889
8				111	92	113	436	400		7065	3193	4492
9				111	90	95				6008		5213
10				110	93	110	433		278	4782	3152	4795
11				110	92	93	413	376	270	4876	3053	4762
12						94	385		275	6222	3551	4552
13						91				4398	5452	5237
14						90	255	295	217	4273	4050	
15						98	243	277	200	4050	3431	
16							257	244	220	3936	3727	4172
17							253	243	220	3728	3843	4239
18								250		3727		4300
19								247		3830	2826	4006
20											2745	3962
21										3721	2632	3989
22										2640	2651	3446
23										2846	2741	3843
24										2683	2699	2680
25												
26										2947	3173	3207

All urine collections were analyzed for total nitrogen by the Kjeldahl method, using mercury and potassium sulfate in the digestion mixture, and for total creatinine (including any creatine present) by a modification of the Folin method already described (Mitchell and Carman, '26 c).

THE RESULTS OF THE EXPERIMENTS

The essential results of the experiments, averaged to save space, are given in table 5 together with statistical measures of the variation of the individual data. The average data from the initial basal metabolism determinations[5] are given and related to the average total endogenous nitrogen excretions, the ratios in the last column being obtained from the two individual measures expressed per m^2 of body surface. The average creatinine excretions are given in various forms and, in the last column, the average ratio of the creatinine N to the output of basal heat, both being expressed on the surface area basis.

The basal metabolism determinations of this study may profitably be compared with some of those previously reported on the same species of animals by other investigators, representing what appear to be the best published determinations on animals in the later stages of growth. All values cited below have been computed from surface areas estimated according to the formulas given on page 419. They are hence directly comparable with one another.

For mice, the reported values are extremely variable, probably because of the difficulty of maintaining quietness for any length of time with this species—a point which Benedict and Fox ('32–'33), in particular, emphasize. However, the average basal metabolism rate obtained in this experiment, 1192 calories per m^2 per day, is considerably higher than that of Benedict and Fox, 726 calories, or the closely agreeing values of Davis and van Dyke ('33), 735 calories, and of Aszódi ('21),

[5] In these basal metabolism determinations, the respiratory quotients averaged 0.72 for mice, rats and guinea pigs, 0.73 for rabbits, and 0.75 for pigs. The individual quotients varied only from 0.70 to 0.75 with two exceptions: for two of the pigs the R.Q. was 0.79.

TABLE 5

Summary of average determinations and ratios for the various species of animals. These results are all expressed on the daily basis

SPECIES	NUMBER OF ANIMALS	BASAL METABOLISM[1]		ENDOGENOUS N[1]		RATIO OF ENDOGENOUS N TO BASAL HEAT PER M²	CREATININE NITROGEN[1]			CREATININE COEFFICIENT	RATIO OF CREATININE N TO BASAL HEAT
		Per kilogram body weight	Per m² body surface	Per kilogram body weight	Per m² body surface		Per kilogram body weight	Per m² body surface	Per cent of total endogenous N		
		cal.	*cal.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*mg.*	*mg.*
Mouse	9										
Average		322	1192	614	2308	1.941 ± 0.030	30.1	113	4.92	81	0.0951
Standard deviation		22.1	62.3	45.6	105.8	0.136	2.53	5.14	0.277	....	0.0035
Coefficient of variation		6.9	5.2	7.4	4.6	7.01	8.4	4.5	5.6	....	3.69
Rat	23										
Average		109	748	213	1481	1.988 ± 0.023	15.1	95.5	7.11	41	0.141
Standard deviation		14.6	88.3	22.1	156	0.161	1.50	14.0	0.800	....	0.0176
Coefficient of variation		13.4	11.8	10.4	10.5	8.10	10.0	13.3	11.2	....	12.48
Guinea pig	9										
Average		95	958	178	1827	1.908 ± 0.034	12.7	131	7.18	34	0.137
Standard deviation		7.21	50.1	14.4	160	0.150	0.61	10.0	0.720	....	0.0125
Coefficient of variation		7.6	5.2	8.1	8.8	7.86	4.8	7.6	10.0	....	9.09
Rabbit	10										
Average		59	777	121	1652	2.128 ± 0.019	17.1	233	14.3	46	0.304
Standard deviation		7.06	115	13.9	256	0.091	1.54	30.8	2.5	....	0.0522
Coefficient of variation		12.0	14.8	11.5	15.5	4.26	9.0	13.2	17.5	....	17.17
Pig	5										
Average		19.6	959	40.4	2006	2.100 ±0.038	7.3	360	18.2	19.5	0.378
Standard deviation		1.85	96	3.6	187	0.128	1.1	60	3.8	....	0.063
Coefficient of variation		9.4	10.0	8.8	9.3	6.10	15.5	16.8	20.9	....	16.69
Grand average						**2.004 ± 0.015**					

[1] Surface areas were estimated from body weights in this table by the following formulas:

Mice $S_{cm^2} = 15.18\ W_{gm.}^{.666}$ (Smuts, '33)
Rats $S_{cm^2} = 12.54\ W_{gm.}^{.60}$ (Lee, '29, Am. J. Physiol., vol. 89, p. 24)
Guinea pigs $S_{cm^2} = 7.35\ W_{gm.}^{.667}$ (Smuts, '33)
Rabbits $S_{cm^2} = 56.33\ W_{gm.}^{.426}$ (Smuts, '33)
Pigs $S_{m^2} = 0.097\ W_{kg.}^{.666}$ (Brody, '28, Mo. Agr. Exp. Sta. Res. Bull no. 115)

746 calories. This is the more puzzling, since none of these investigators claims that his animals were completely quiescent, while in this study the experimental mice were observed to be at absolute rest. A possible explanation is the apparent instability of the temperature-regulating mechanism of the mouse—a phenomenon described in considerable detail by Asźodi.

The basal metabolism values for rats obtained in this experiment, averaging 748 calories per m^2 per day, were quite in line with previously reported values, but show no appreciable sex difference. Thus, Mitchall and Carman ('26 b) have reported results on forty-two rats averaging 777 and 731 calories per m^2 for male and female rats, respectively, while values of Benedict and MacLeod ('29 b) on seventeen rats average 798 and 741 calories, respectively. The values for guinea pigs, averaging 958 calories per m^2, agree well with the results of Murschhauser and Hidding ('12) on four animals, averaging 902 calories, but are somewhat higher than the average reported briefly (148 animals) by Siebert and Thurston ('31–'32), i.e., 856 calories. This is the lowest average value reported in the literature; it seems possible that the subjection of the experimental animals in these studies to a basal metabolism test three to four times a week may have depressed the value to subnormal levels.

The conditions for the determination of the basal metabolism of rabbits have never been defined, in so far as the author is aware. None of the published results of such work specifies the environmental temperature imposed, and none but that of Wiegner and Ghoneim ('30–'31) can, in view of the tests on this point described above, be considered to be truly post-absorptive values. The average value of the ten secured in this experiment, 777 calories per m^2, approximates that of Wiegner and Ghoneim, 728 calories. That it also approximates other results which were obtained after much shorter periods of fasting, may be traced to the peculiar fact noted above, namely, that a level of metabolism similar to the true post-absorptive level is reached after only 24 hours of fast and possibly somewhat earlier.

The basal metabolism of the five pigs in this experiment averaged 959 calories per m^2 per day—a somewhat lower value than any previously reported, although it approximates the one result of Capstick and Wood ('22), 1027 calories, and the later results of Deighton ('29) on two pigs, averaging 1079 calories. From Brody's work ('32 b, table 8) female pigs, weighing 75 pounds or more, were found to have basal metabolic rates averaging 1026 calories per m^2 per day. There may be some question whether these values represent the true post-absorptive conditions, because of the shortness of the fasting period.

The plan of the experiment contemplated a second basal metabolism determination upon all animals at the conclusion of the period of low nitrogen feeding, but a complete second series of such measurements was made only in the case of the male rats and the guinea pigs. For the rats, the second series of results checked well with the first (1 per cent average difference), indicating that the basal metabolic rate was not disturbed by this abnormal dietary regime. For the guinea pigs, however, a consistent rise in basal metabolism was observed, averaging about 10 per cent.

For most animals, published measurements of the endogenous nitrogen output are few, if any at all have been reported, and in this connection the many results of Terroine and associates cannot, unfortunately, be used, because his so-called 'endogenous nitrogen' includes both urinary and fecal nitrogen on a nitrogen-free diet.

From a compilation of 450 determinations of the endogenous nitrogen of rats, mainly from the Division of Animal Nutrition of the University of Illinois, but also from other laboratories in this country and Europe, an average value of 1238 mg. of nitrogen per m^2 of body surface was computed. The rats varied in weight from less than 30 gm. to 450 gm. There was an apparent tendency for this ratio to be somewhat higher for the heavier rats, averaging about 1430 mg. for rats weighing 150 gm. or more. This value is very close to the average obtained in this study of 1481 mg. for both male and

female rats. Ashworth and Brody's voluminous data ('33 a) on the endogenous nitrogen excretion of rats were obtained intermittently during prolonged periods of feeding on low-nitrogen diets. Such data in their entirety are not comparable with the data reported in this paper, since it is probable that the excretion of nitrogen in the urine after such prolonged feeding of this character does not represent the endogenous level of nitrogen metabolism any more than the heat production after prolonged fasting represents the basal metabolism. Hence, the low levels of nitrogen output reported by the Missouri investigators, averaging only about 1000 mg. per m^2 of body surface, are not surprising in view of the long periods of specific nitrogen starvation. Unfortunately, in the tables presenting their data, no information concerning the time at which ration changes were made is given, thus precluding the selection of such of their data as were obtained under conditions comparable to those adopted in this study.

The average excretion of endogenous nitrogen found for the rabbit, 1652 mg. per m^2 of body surface, is higher than the few determinations of this quantity reported in the literature. Mendel and Rose ('11–'12) fed one rabbit on sugar for a sufficient period of time to attain a constant level of nitrogen output, averaging 1466 mg. per m^2 of body surface. A somewhat similar study was conducted by Myers and Fine ('13) on one rabbit. During a 7-week period of carbohydrate feeding, the total urinary nitrogen output averaged 1276 mg. per m^2 of body surface. In a report by Serio ('23), data are given on the urinary nitrogen output of a rabbit during a period of low-nitrogen feeding. For the last 6 days of this period the excretion averaged 1313 mg. per m^2. Although these three results on the rabbit are less than the average excretion obtained for the ten rabbits in this study, they fall essentially within the range of the individual results, i.e., 1278 to 2015 mg.

The average of 2006 mg. of endogenous nitrogen per m^2 of body surface for five pigs may be compared with an average of 1844 mg. for five pigs of similar weight from experiments

of McCollum and Steenbock ('12) and McCollum and Hoagland ('13), and an average of 2297 mg. for eight pigs from the experiments of Mitchell and Kick ('27).

Determinations of the endogenous urinary nitrogen excretions for the mouse and guinea pig, with which to compare those made in this study, have not been found in the literature. Such comparisons as have just been made are of value in judging the validity of the relations between basal metabolism and endogenous nitrogen metabolism that will be discussed in the next section of this report.

DISCUSSION OF EXPERIMENTAL RESULTS

From the averaged data of table 5, it is evident that both the basal metabolism and the endogenous nitrogen excretion are much more closely related to body surface than to body weight, since, when expressed to the unit of body surface, much less variable values are obtained among the different species than when expressed to the unit of body weight. Also, it is evident that a marked correlation exists between the two measurements, so that the ratio of endogenous nitrogen in milligrams to basal calories is remarkably constant among the five species of animals studied. The average ratio is approximately 2.00 mg. of endogenous nitrogen excreted for every calorie of basal heat produced. While the differences in average ratio between some of the species, namely, between rabbits and rats, mice, or guinea pigs, and between pigs and guinea pigs or mice, are statistically significant, this fact is due more to the great constancy of the ratio within each species rather than to any great disparity between average specific ratios.

The variability of the ratio within each species is well measured by the coefficient of variation. These coefficients are 7.01 for mice, 8.10 for rats, 7.86 for guinea pigs, 4.26 for rabbits and 6.10 for pigs. These degrees of variability may be compared, for example, with the variability in the basal heat production of adult human beings, expressed per m^2 of body surface. As calculated by Harris and Benedict ('19, p. 147) for different series of observations, the coefficient of variation

ranges from 6.75 to 11.13, except for a small series of measurements on athletes, for which the coefficient of variation was 3.97.

The most striking confirmation of the close correlation existing between the basal metabolic rate and the endogenous excretion of nitrogen is afforded by the data on rats and rabbits. The animals of these two species selected for the experiment were in quite different nutritive states, some having been taken from other experiments—this being true of all of the rabbits—in which different conditions were imposed, and some having come directly from the stock colony. As a result, they were the most variable groups, both with reference to the basal metabolism and to the excretion of endogenous nitrogen, as the coefficients of variation (table 5) indicate. But in spite of this great variability in these two factors among the rats and the rabbits, the ratio between them was not unusually variable; in fact, for the rabbits the ratio was more nearly constant than for any other species.

The percentage deviations of the individual ratios of endogenous nitrogen to basal calories from the average ratio for all animals, 2.00, average 6.86. Seventy-seven per cent of these percentage deviations are no greater than 10, and 93 per cent of them are no greater than 15. The coefficient of variation of the individual ratios about the mean of all is 8.12. The ratio of endogenous nitrogen to basal metabolism thus appears to be as constant within these five species of animals as the ratio of basal metabolism to body surface is within the human species, and far more constant than the latter ratio among different species.

The applicability of this very constant ratio of 2 mg. of endogenous nitrogen for each calorie of basal heat to the human species may be tested from the data of two investigations in which the experimental subject was submitted to both measurements. Smith ('26), in his study of the endogenous nitrogen metabolism, also measured the basal metabolism of his subject twice during the period of low-nitrogen feeding. The first determination was made on the

thirteenth day of this regime, on which day the subject was producing 1383 basal calories and excreting 2880 mg. of urinary nitrogen, representing a ratio of 2.08 mg. of nitrogen per calorie. At this time, contrary to the experience of Folin and others, the urinary nitrogen had not yet reached a constant level, and shortly after this time its rate of excretion diminished rapidly to the twenty-fifth day of low-nitrogen feeding, when the second basal metabolism determination was made. The ratio of endogenous nitrogen to basal heat now was only 1 mg. per calorie. Two years later, Deuel, Sandiford, Sandiford and Boothby ('28) published their study of the nitrogen minimum in man, throughout which frequent determinations of basal metabolism were made. The first period of this experiment consisted of 30 days of low-nitrogen feeding, and is the only period that need be considered in this connection, since in the later periods conditions were imposed that would be expected to induce rates of excretion of urinary nitrogen bearing no known relation to Folin's endogenous metabolism. On the ninth day of specific nitrogen starvation, the urinary nitrogen excretion had attained an approximate level, and the ratio of this excretion to the basal heat was 1.96 mg. per calorie. For the next period of 7 days, the ratio was 2.21, and for the next period of 6 days it was 1.95. After this time the ratio decreased to 1.44 mg. per calorie.

These two experiments, and particularly the latter, constituting the only published evidence known to the writer, indicate that in the human species, as in the lower animals, the endogenous nitrogen output bears a ratio to the basal heat approximating 2.0 mg. to the calorie. They also indicate that protracted feeding on low-nitrogen diets may disturb this ratio by depressing the endogenous nitrogen output, just as protracted fasting depresses the basal metabolism. Possibly the erratic and generally low ratios reported for rats by Ashworth and Brody ('33 a) are traceable to the variable, but generally long periods of low-nitrogen feeding.

The relation between the creatinine excretion and the basal metabolism for the fifty-six animals of this experiment is also

set forth in table 5. Within any one species the ratio of creatinine nitrogen to basal calories is fairly constant, as Palmer, Means and Gamble ('14) found for humans, this being particularly true for mice, rats and guinea pigs. But among the different species, the average ratios increase in size with size of animal, being 0.095 mg. per calorie for mice, 0.141 mg. for rats, 0.137 mg. for guinea pigs, 0.304 mg. for rabbits, and 0.378 mg. for pigs, this being in general agreement with the experimental results of Terroine and Garot ('26). Among rats a distinct sex effect is evident, the ratios of the males being distinctly higher than those of the females, the averages being, respectively, 0.148 and 0.129 mg. of creatinine nitrogen per calorie of basal heat.

The inconstancy of the ratio of creatinine to basal heat in interspecific comparisons, in spite of the constancy of the ratio of total endogenous nitrogen to basal heat, is the result of specific differences in the percentage of endogenous nitrogen represented in the creatinine excretion. This percentage is low for the mouse, 4.92, intermediate for the rat and guinea pig, 7.11 and 7.18, and high for the rabbit and pig, 14.3 and 18.2, showing a positive correlation with body size. In seeking an explanation of these interesting relationships, reference may be made to the data collected and compiled by Grafe, Reinwein and Singer ('25), with reference to tissue respiration when in situ and when removed from the body. From their own data and from data compiled from the literature, they show that similar tissues from different warm blooded animals respire at about the same rate (expressed as oxygen consumed per gram of dry matter per minute) when removed from the body, although in situ they respire at markedly different rates in rough accordance with the surface area law. Furthermore, only in the small animals, such as the mouse, is the rate of respiration of the intact tissue as great as that of the excised tissue. The larger the animal, the greater the depression of tissue respiration with reference to that of the isolated tissue.

From the results of this experiment one may reasonably infer that there is in all warm blooded animals a constant (in the biological sense) ratio between the basal tissue respiration and the basal catabolism of the nitrogen-containing tissue constituents. In small animals, such as the mouse, in which the tissue respiration is the same whether in situ or isolated, the metabolism of creatine accounts for only a small fraction of the total endogenous nitrogen metabolism. In larger animals, in which the rate of tissue respiration in situ becomes increasing smaller than that of the excised tissue, the creatine metabolism assumes a larger and larger role in the total endogenous nitrogen metabolism. In other words, the depression of tissue oxidation in large animals, brought about by some unknown regulatory mechanism the operation of which results in approximate conformance to the surface area law, is accompanied by a parallel depression of the endogenous catabolism, but the greater burden of this latter depression is borne by the non-creatine constituents of the tissues. Whether this means a greater depression of respiration in the non-muscular tissues than in the muscular tissues is an interesting question.

The results of this investigation, together with the work of Terroine and associates, afford a promising method of estimating the maintenance requirements of protein for different animals from the basal metabolism. According to this method, 2 mg. of nitrogen, or 12.5 mg. of conventional protein, are required daily for each calorie of basal heat. Since basal metabolism varies with body surface rather than with body weight itself, it may be concluded that the protein requirements for maintenance vary likewise, and that the current method of expressing such requirements per unit of body weight is definitely in error. While the investigation reported here was concerned mainly with adult animals, Sorg-Matter ('28) found that age did not disturb the relationship between endogenous nitrogen and basal heat, so that, until evidence to the contrary is forthcoming, it seems justifiable to apply the above ratio to warm blooded animals of all ages.

Brody ('32 a) has shown that the basal metabolism of mature mammals and of the domestic fowl may be predicted by the following equation

$$Q = 70.4\ m^{.734}$$

where Q is the basal heat in calories per 24 hours and m is the body weight in kilograms. This equation may be adapted to a determination of the protein requirements by the use of the ratio of 12.5 mg. of protein per calorie of basal heat. The equation then becomes

$$P = .88\ m^{.734}$$

where P is the day's requirement of protein expressed in grams.

It should be understood that this expression of the protein requirement for maintenance refers not to dietary protein, nor to digestible protein, but to protein actually utilized in the replacement of endogenous losses. The corresponding amount of dietary protein would be larger than this, and would be greater, the greater its wastage in metabolism and hence the smaller its biological value. A biological value of 50 may be regarded as a safe one to use in estimating what may be considered an average requirement of digestible protein. Hence, the net requirement of protein, as computed from the above equation, when multiplied by 2 will give an estimate of the requirement of digestible protein of less than average quality. In fact, when even poor protein mixtures are fed at the low level required in a maintenance ration, they should be much better utilized than 50 per cent, so that the margin of safety in this proposed estimate of the maintenance requirement of protein seems ample.

How do the requirements computed in this manner compare with the requirements estimated by the methods of Sherman ('20) and of Armsby ('17)? These methods agree in assigning 0.6 part of digestible protein per 1000 parts of live weight, the Sherman method relating only to humans, while Armsby's method relates to domesticated farm animals of varied sizes. The comparison is given in table 6 for animals ranging in size from 100 gm. to 681 kg.

It is evident that for animals heavier than about 150 pounds, the proposed method gives lower estimates than the other methods. At 150 pounds the estimates are very nearly the same, and at lower weights, the method here proposed gives higher estimates. The lower proposed estimates for the heavier animals is in line with the well-known liberality of the Armsby standard for such animals as beef cattle and dairy cattle, while the higher proposed estimates for very small animals seem obviously nearer the truth. For example, a 200 gm. rat has a basal metabolism of 22.5 calories daily

TABLE 6

A comparison of methods of estimating the requirements of animals for digestible protein for maintenance

BODY WEIGHT OF ANIMAL		DIGESTIBLE PROTEIN REQUIRED ACCORDING TO			
		PROPOSED METHOD		THE METHOD OF ARMSBY (AND OF SHERMAN FOR MAN)	
kg.	*lb.*	*gm.*	*lb.*	*gm.*	lb.
681	1500	211	0.465	409	0.90
454	1000	157	0.35	272	0.60
227	500	94	0.208	136	0.30
91	200	48	0.106	54	0.120
68	150	39	0.086	41	0.090
45	100	29	0.064	27	0.060
2.2	5	3.15	0.007	1.36	0.003
0.2	0.44	0.540		0.120	
0.1	0.22	0.325		0.060	

and would require for maintenance of body weight approximately 30 calories of gross food energy (Mitchell and Carman, '26 a) daily, or 7 gm. of food with an energy value of 4.25 calories per gram (also, Mitchell, '24, p. 880). If this ration contains 4 per cent of a high quality protein, such as egg protein, it will slightly more than support nitrogen equilibrium, according to the experience of this laboratory. Seven grams of such a ration will contain 280 mg. of protein. But egg protein at this level seems to be completely utilized in metabolism (Mitchell and Carman, '26 c). For a protein with a biological value of 50, assumed for the estimates in

table 6, 560 mg. of protein (280 × 2) would be required. The estimate given in the table for a 200 gm. animal is 540 mg., while the Armsby standard prescribes only 120 mg.

The estimates in table 6 relate only to mature animals, since the Brody equation on which they are in part based is so restricted. To estimate the requirements of protein for the maintenance of young growing animals, the Brody equations relating basal metabolism to body weight for different species (Brody, '32 b) may well be used.

CONCLUSIONS

Among the species of animals investigated (mouse, rat, guinea pig, rabbit and pig), the smaller the body size the more rapid is the adjustment to the post-absorptive condition during fasting, and the more rapid is the adjustment to the endogenous level of nitrogen excretion in the urine during specific nitrogen starvation.

Among warm blooded animals, the total endogenous nitrogen output—and hence the protein requirement for maintenance—varies more nearly with the body surface than with the body weight.

There is a close relationship between the total endogenous nitrogen excretion of warm blooded animals varying widely in size and their basal metabolism, such that 2 mg. of nitrogen are lost to the body daily for every calorie of basal heat. This relationship appears to apply also to the human species. The endogenous catabolism of an animal, regardless of species, may be estimated in this manner from its basal heat production as accurately as the latter may be estimated (under the best conditions) from its body surface or body weight. This estimate of the endogenous loss of nitrogen is the basic information necessary to compute the maintenance requirement of protein.

Although within any species a fairly constant relationship exists between the excretion of creatinine and the basal metabolism, there is no constant relationship among different species in this respect, because for different species the

creatinine nitrogen characteristically accounts for different percentages of the total endogenous nitrogen. The smaller the body size in a species, the smaller the percentage of the total endogenous nitrogen represented in the creatinine output.

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RATES OF ABSORPTION AND GLYCOGENESIS FROM VARIOUS SUGARS

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THREE FIGURES

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The question of the rate of absorption of carbohydrates from the gastro-intestinal tract is intimately related to any problem of glycogen synthesis. Cori ('25) concluded that the rate of absorption of hexoses was independent of the absolute amount and concentration of the sugar in the intestine, giving a straight line relationship between the absorption rate and time. Magee ('30) states that Auchinachie, Macleod and Magee in unpublished work had, in general, confirmed Cori's results for 'sugar.' Wishnofsky ('34), using fourteen human female diabetics, also reached the conclusion that the rate of absorption of dextrose from the human gastro-intestinal tract was the same, regardless of the absolute amount present and affirmed as a corollary to this that the rate of absorption of dextrose was constant. We feel that for the data presented only one of the three groups in his table 1 warrants such a conclusion. Pierce, Osgood and Polansky ('29) concluded that there was a falling off in the rate of absorption during the second and especially the third hour after feeding. Cori, Cori and Goltz ('29) pointed out that Pierce et al. did not feed enough sugar to enable absorption to proceed for 3 hours. Since 1929, results from several laboratories have been published showing that the rate of glucose absorption is not uniform as stated by Cori, but tends

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to decrease with time. Burget, Moore and Lloyd ('32) state, "The average results of our experiments on rats show a decided decrease in absorption during the second hour." These workers did not study absorption rates after 2 hours. Ravdin, Johnston and Morrison ('33), using fox hounds with jejunal loops, state, "When the amount of sugar absorbed is plotted against time, no linear relationship can be demonstrated." Burget, Moore and Lloyd ('33), studying absorption of glucose by chronic loops of colon, present data which lead us to infer that there is a more rapid absorption during the early than during the late stages of a 90-minute period, although it is difficult to tell whether the data for the periods in their table 2 are cumulative or differences for successive periods. MacKay and Bergman ('33 a, '33 b), using rats, studied the rate of absorption of glucose in relation to the previous diet and found a falling off in the absorption rate in successive hours. Trimble, Carey and Maddock ('33), utilizing Cori's technic with dogs, found no definite relationship between variations in the concentration or absolute weight of glucose ingested and the rate of absorption. As MacKay and Bergman ('33 b) have pointed out, this is apparently true under the particular conditions of their experiments. The glucose fed was increased the longer the absorption period, thus 2.12 gm. glucose per kilo were given to dogs for the 1-hour period, 3.21 gm. per kilo for the 2-hour period, and 4.26 gm. per kilo for the 3-hour period. It seems likely that the sustained average rate of absorption was due to the increasing amounts of glucose fed.

Evidence is accumulating that the concentration of the glucose solution fed is related to the amount of glucose absorbed. Results reported by MacKay and Bergman ('33 b) and Ravdin and his associates ('33 a) show that by feeding increasing amounts of glucose or solutions of high concentration, the rate of absorption is increased.

Adam ('24) using infants, and Holtz and Schreiber ('30) using dogs, observed, by use of x-rays, the movement of sugar solutions containing either $BaSO_4$ or colloidal SiO_2 through

the alimentary tract. Their data reveal the importance of the emptying time of the stomach as related to possible effects upon sugar absorption.

Ravdin and his associates ('33 b) have presented results showing how sugar solutions of varying concentration are diluted to approximately the same percentage composition prior to their absorption from the intestine when such solutions are fed as described by Cori. Thus, the sugar solution coming into contact with the absorbing surface of the intestine, having approximately the same concentration irrespective of that of the original solution fed, should not cause much change in the absorption rate where the whole alimentary tract is used.

In whichever manner absorption is studied, it is clear from the large variations obtained by Cori and associates, by MacKay and Bergman and by ourselves, that a large number of animals must be used to determine a dependable average and that the coefficients of variation must be given.

OBJECT

The object of the present investigation was twofold a) to redetermine the rate of absorption of glucose from the alimentary tract of rats; b) to compare this rate with that of other sugars requiring some digestion and to determine the relative rates of liver glycogen formation by these sugars; namely, a simple sugar, d-glucose (dextrose[2]), a double sugar, sucrose and a complex of single, double and multiple sugars, such as pure corn syrup.[3] The rate of glycogenesis was then studied in relation to the rate of absorption.

METHODS

In part a) of this study rats weighing between 120 and 270 gm. were used as experimental animals, seventeen of the 130 animals being females. The greater proportion ranged

[2] Supplied by the Corn Industries Research Foundation and confirmed as to purity by polariscope.

[3] Supplied by the Corn Industries Research Foundation and confirmed as to yield of total dextrose by hydrolysis.

from 150 to 200 gm. in weight and were selected from a colony of inbred Wistar stock available in the laboratory. Approximately half of the animals received the stock diet mentioned by Pierce, Osgood and Polansky ('29), while the others had been fed on the standard stock diet of Purina dog chow since the time of weaning.

In part b) adult male rats weighing from 150 to 300 gm. were used both for absorption and formation of glycogen in the liver. They were selected from the same colony of inbred Wistar stock. All had been fed on the standard stock diet of Purina dog chow since the time of weaning, thus reducing to a minimum differences in glycogenesis which might possibly be due to the effect of the previous diet. The sugars were compared from the standpoint of equivalent quantities administered, and a 50 per cent solution of dextrose was used as the standard. Sucrose (Pfanstiehl) was therefore fed in a 47.5 per cent solution, and corn syrup was prepared in a solution equivalent to the 50 per cent dextrose solution from a knowledge of its percentage composition, confirmed by acid hydrolysis.

TABLE 1

Composition of corn syrup

	DRY BASIS	MOIST BASIS
	per cent	*per cent*
Dextrins	56	45
Maltose	27	21.7
Dextrose	16	12.9
Ash	0.25	0.21

Total milligrams carbohydrate per gram syrup = 796

In part a) of this investigation, the rats were fasted for 24 hours and then, with but few exceptions (when 2.0 cc. were administered), fed 2.5 cc. of a 50 per cent dextrose (Merck) by stomach tube using the Cori ('25) technic. At the end of 1-, 2- and 3-hour intervals the different animals were killed by a blow on the head, the alimentary tract removed and washed, and glucose determined in the washings by the Bertrand method (Mathews, '25). All precautions cited formerly

(Pierce, Osgood and Polansky, '29) were observed. All of the data are taken from results obtained by one of us in person or by selected groups of medical students working under his immediate direction (series A, table 2). The results shown under series B were those obtained by the other author (F) working independently, while those in series C were obtained in an earlier study (Pierce, Osgood and Polansky, '29). These results are republished so that they may be compared with those of the present study, and also for the purpose of showing the effects of a 48-hour as compared with a 24-hour fast on the absorption coefficients.

In part b) the experimental animals were also fasted for a period of 24 hours, and then fed 2.5 cc. of the various carbohydrate solutions to be tested by stomach tube. At the end of 1-, 2- and 3-hour intervals, the animals were killed by a blow on the head, the livers quickly excised, the gastro-intestinal tract quickly ligated at the cardia and ileo-cecal valve, then removed, washed and the unabsorbed sugar determined.

From the data thus obtained it was possible to determine the rate of absorption of glucose in comparison with more complex sugars, as well as the relative rates at which glycogen was deposited in the tissues of the liver.

RESULTS

Absorption studies

1. Absorption of glucose. Table 2 presents a summary of the results obtained in studying the rate of absorption of glucose with three groups of rats. Series A was designed especially to supply a larger body of data than any hitherto published. To meet the criticism of our original results by Cori, Cori and Goltz ('29), larger amounts of sugar were fed to insure the presence of sufficient sugar to permit the same sustained rate of absorption as was taking place during the 1-hour absorption period. In only three of the thirty-seven rats used in series A for the 3-hour absorption period was there insufficient sugar to permit a rate of absorption as great

TABLE 2

Summary of the rate of absorption of d-glucose from the gastro-intestinal tract of rats

NUMBER ANIMALS	WEIGHT AFTER FASTING 24 HOURS	GLUCOSE			GLUCOSE ABSORBED MILLIGRAM PER HOUR PER		BASIS BODY WEIGHT		BASIS SQUARE CENTIMETER BODY SURFACE		GLUCOSE ABSORBED
		Fed	Absorbed	Unabsorbed	100 gm. body weight	Square centimeter body surface	Standard deviation	Coefficient variability	Standard deviation	Coefficient variability	
					Series A						
					1-hour						
	gm.	*mg.*	*mg.*	*mg.*				*per cent*		*per cent*	*per cent*
56	177	1268	365	903	213	1.295	70.7	33.19	0.403	31.12	29.09
					2-hour						
37	195	1160	690	470	181	1.145	47.4	26.19	0.257	22.43	59.30
					3-hour						
37	183	1149	859	290	160	0.996	44.6	27.88	0.240	24.10	74.9
					Series B						
					1-hour						
8	207	814	513	301	246	1.601	116.1	47.2	0.605	37.8	63.4
					2-hour						
9	200	743	554	189	143	0.894	23.0	16.1	0.129	14.6	76.3
					3-hour						
29	218	954	802	152	127	0.822	25.0	19.7	0.129	15.7	84.0
					Series C fasted 48 hours						
					1-hour						
9	169	731	381	350	236	1.40	59.3	25.13	0.289	20.60	52.6
					2-hour						
7	152	887	705	182	229	1.37	26.0	11.35	0.107	7.82	79.4
					3-hour						
7	157	857	788	69	168	0.99	14.6	8.69	0.078	7.85	91.9

as that noted in the 1-hour period. In all of the other animals there was more than enough glucose to permit the higher rate of absorption obtained during the first period. It will be noted from the data in table 2 under series A that an average of 290 mg. glucose remained unabsorbed after 3 hours. The condition in series B is somewhat different, for here, if absorption were to continue at the rate noted during the 1-hour period, over 1000 mg. of glucose would have had to be fed in place of the 743 mg. given. In spite of this fact, and with 189 mg. glucose remaining after 2 hours of absorption, the coefficient had dropped to 143. Certainly, there was sufficient glucose remaining to have made this value higher, as is true also for the third hour, if absorption were independent of amount present. The data in series C indicate that absorption of glucose after 48 hours' fasting is more uniform from animal to animal than after 24 hours' fasting.

Our calculation of glucose absorption as set forth in the first paper (Pierce et al., '29) has also been criticized by Cori and his associates ('29) and data presented in their table 1 show our calculations as not agreeing with their results. According to one of Cori's ('25) earlier conclusions, 50 per cent glucose is absorbed from the intestine at a constant rate. If this were true, it could be assumed that the data presented in the paper by Cori et al. ('29) for the different periods were interchangeable. Using data in their table 1, suppose we place the figures given under the column headed '0–2 hours' under the column headed '0–1 hour' and calculate the results on the basis of 1 hour of absorption instead of 2. Similarly, transpose the figures under the heading '0–3 hours' to column headed '0–2 hours' and calculate results on the basis of 2 hours' absorption instead of 3, and, finally, place the figures under the column headed '0–1 hour' under that headed by '0–3 hours' and recalculate on the basis of 3 hours. The averages given in table 1 of Cori et al. ('29) are presented in our table 3, as are the results obtained on transposing and recalculating the data in the manner just described. It can be seen that the results predicted by us are not far from those

obtained by interchanging Cori's data, and the variation is not exceptional for such experiments, as may be seen from the coefficients of variability given in table 2.

Upon examining our data under series A, table 2, and calculating results on the basis of a constant percentage rate, we find an absorption of 29.1 per cent (365 mg.) of glucose

TABLE 3

Showing relation of Cori's results as obtained on transposing data as described in text

	0 TO 1 HOUR			0 TO 2 HOURS			0 TO 3 HOURS		
	Absorbed	Average absorbed coefficient	Absorbed	Absorbed	Average absorbed coefficient	Absorbed	Absorbed	Average absorbed coefficient	Absorbed
	mg.		*per cent*	*mg.*		*per cent*	*mg.*		*per cent*
Data presented by Cori et al. 1929, table 1	203	203	21.7	469	235	50.5	660	220	70.9
Expected according to Pierce[1]				363	182	38.6	489	163	51.9
Transposing and recalculating as described in text	235	235	25.2	440	220	46.3	609	203	64.6
Expected according to Pierce				415	208	43.7	548	183	58.1

[1] Calculation: Quoting from Cori et al ('29). Four rats each killed 1, 2 and 3 hours after glucose feeding. The average amounts fed per 100-gm. rat were 943 mg. for the 1-hour, 931 mg. for the 2-hour and 951 mg. for the 3-hour absorption period. The average body weight of the three groups of rats was 140.5, 142.6 and 138.2 gm., respectively. Since 21.7 per cent of 943 is absorbed in the first hour, 740 mg. of sugar are left in the intestine after 1 hour; 21.7 per cent of 740 is supposed to be absorbed in the second hour, leaving 578 mg. for the third hour, and so on.

during the first hour, which leaves 70.9 per cent (903 mg.) remaining in the alimentary tract. If 29.1 per cent of the remaining 70.9 per cent were absorbed during the second hour, 20.6 per cent (262 mg.) would be taken up, making the total 49.7 per cent (631 mg.) as against the value of 59.3 per cent (690 mg.) which was found. Finally, if 29.1 per cent of the remaining 50.3 per cent (637 mg.) were absorbed during the third hour, 14.6 per cent (185 mg.) would be taken up,

making the total absorption 64.3 per cent (815 mg.) in place of 74.9 per cent (859 mg.) which was found. These values are markedly different from those reported previously (Pierce, Osgood and Polansky, '29) and do not support our former belief that the amount of glucose absorbed at all times is a uniform percentage function of the concentration of glucose remaining in the alimentary tract. This does not lead us to conclude, however, that glucose concentration is a negligible factor, as will be shown in the following paragraph.

If we take the results in our present table 2, those of Pierce, Osgood and Polansky ('29) in their table 1, of Cori ('25) in his table 2, of Cori et al. ('29) in their table 1, and of MacKay and Bergman ('33) in their table 2, experiment 1-C, and plot the average amount of sugar available for absorption against the average sugar absorbed per 100 sq.cm. of body surface at 1, 2 and 3 hours, there is a definite slope to the curves showing a lowered absorption with decreasing amounts (fig. 1). All data plotted, with the exception of the very few of Cori et al. ('29) show a lessened absorption with a falling concentration. The agreement in different series is perhaps as good as could be expected. It is likely that the emptying time of the stomach is affected by its degree of filling and the relative filling of the intestine in turn by this emptying time. Absorption must be affected by the amount of mucosal surface exposed to the sugar.

2. Absorption of different sugars. In table 4 and figure 2 are shown the summaries of results obtained with respect to the relative absorption rates of the three carbohydrates studied in series B. The rates of absorption have been considered on the basis of absorption coefficients each of which represents the number of milligrams of sugar absorbed per 100 gm. of rat per hour. The absorption did not take place at a constant rate for the three successive hourly periods for any of the sugars. In the case of dextrose, the absorption coefficient is decreased approximately 42 per cent during the second hour of the absorption period, while during the third hour it drops to almost one-half of its initial value. The

absorption rate of sucrose follows a somewhat similar course to that of dextrose, although it does not take place at as rapid a rate as does the simple hexose. In all 3 hours, although the marked decrease follows the general course evidenced by the dextrose, all values in terms of body weight are

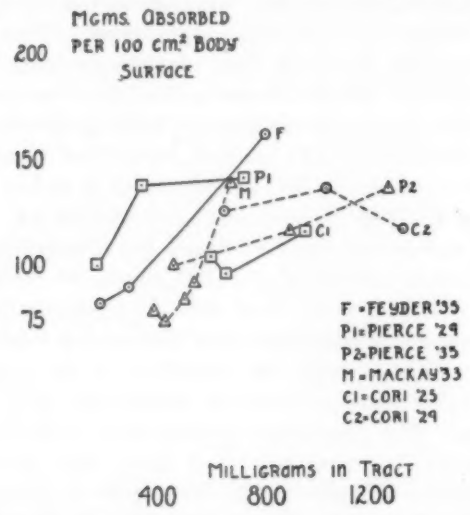

Fig. 1 Showing the average amount of sugar available for absorption plotted against the average sugar absorbed per 100 sq.cm. of body surface at 1, 2 and 3 hours. Pure Merck's dextrose was used by Pierce et al. Feyder (series B, table 2) used pure dextrose from corn. Other authors state only that 'pure glucose' was used.

TABLE 4

Summary of the rates of absorption of dextrose, sucrose and corn syrup from the gastro-intestinal tract

SUGAR	AMOUNT FED	HOURLY RATE IN TIME SHOWN AVERAGE ABSORPTION COEFFICIENTS (MG./100 GM. BODY WEIGHT)					
		Number animals	1 hour	Number animals	2 hours	Number animals	3 hours
	cc.						
50 per cent dextrose	2.5	8	246	9	143	29	127
47.5 per cent sucrose	2.5	14	212	7	119	27	108
Equivalent corn syrup	2.5	11	207	9	148	15	97

slightly below those of the simple sugar. The absorption of corn syrup from the gastro-intestinal tract is surprisingly high in comparison with the other sugars, considering that dextrins make up 56 per cent (table 1) and maltose 27 per cent of its weight on the dry basis. At the second hour it is actually slightly higher in relation to weight than either dextrose or sucrose and at the third hour it is only a little behind sucrose (fig. 2). Digestion of the dextrins evidently proceeds at times quite as rapidly as that of sucrose. Its rate of absorption is decreased approximately only 26 per

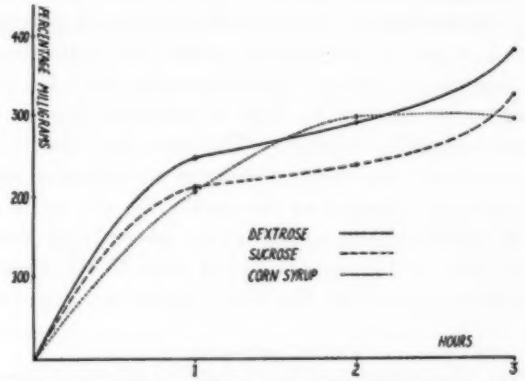

Fig. 2 Average total absorption of rats killed after 1, 2 and 3 hours.

cent during the second hour as compared with 42 per cent for the solutions of dextrose and sucrose, but during the third hour the rate again falls to less than one-half of its initial value. There is no support in these comparative data for Cori's idea of constant rates of absorption of different sugars.

Glycogen studies

The rate at which various sugars and carbohydrate complexes are converted into liver glycogen is a problem which has been investigated by a relatively small number of workers, most of whom have limited their investigations to a study of the simple hexoses and pentoses. It has been found, in

general, that the rapidity of liver glycogenesis from various carbohydrates varies considerably with the carbohydrate administered. Cori ('26) found that when glucose, fructose or galactose were fed to animals previously fasted for 48 hours, and a group of animals killed each hour thereafter for the determination of liver glycogen, curves could be constructed for each sugar to represent the rate of glycogen formation in the liver. The curve for glucose was S-shaped, since there was relatively slow deposition of glycogen during the first 2 hours, and a marked falling off after 4 hours with a rapid deposition intermediate. Cori claims that these variations were not due to an unequal absorption of glucose from the intestinal tract of the animal, since absorption remained constant from hour to hour. The glycogen curve for fructose, Cori found to be similar to that of glucose, except that all values were slightly higher. During the absorption of galactose, he found that liver glycogen was formed one-third as rapidly as from glucose or fructose. On the basis of comparisons in which the inequalities in absorption were duly considered, Cori ('31) has concluded that of the above three sugars fructose is by far the best producer of glycogen in the liver.

EXPERIMENTAL

From all the rats used for the absorption studies in series B (table 2) the livers were taken a moment after killing and plunged into hot 30 per cent KOH for determination of glycogen, according to the method of Good, Kramer and Somogyi ('33). A control series killed after fasting 24 hours and without feeding were treated in an identical manner in order to establish a basal level of glycogen in the livers. An additional series were fed a mixture of equivalent amounts of glucose and fructose for a purpose which will be explained presently.

RESULTS

Table 5 shows the liver glycogen formation of the series of control animals after a 24-hour fasting period and of the other series similarly fasted and 1 hour, 2 hours and 3 hours

after feeding. The glycogen content of the liver has been expressed on the basis of three units of reference, namely, body weight, surface area and liver weight. The average value of 1.78 mg. of liver glycogen per 100 gm. of body weight demonstrates the low basal level of liver glycogen after a fasting period of this length. Computed on the basis of surface area, this value is 1.15 mg. per 100 sq.cm. of surface area. The standard deviation computed on the basis of surface area is lower than that computed on the weight basis, but this is due to the fact that the values are also proportionately smaller. Surface area in all cases has been computed by means of Rubner's modification of the Meeh formula (Diack, '30) which is:

$$\text{Surface area in cm}^2 = 9.13 \times W^{2/3} \text{ in grams}$$

TABLE 5

Liver glycogen formation

SUGAR FED	NUMBER EXPERIMENTS	WEIGHT RANGE	TOTAL IN TIME SHOWN DIFFERENCE BETWEEN CONTROLS (24-HOUR FAST) AND EXPERIMENTALS						
			Per 100 gm. body weight	Standard deviation	Coefficient variability	Per 100 cm.² surface	Standard deviation	Coefficient variability	Per 100 gm. liver
24-hour fast									
		gm.	*mg.*	*mg.*	*per cent*	*mg.*	*mg.*	*per cent*	*mg.*
None	12	191–253	1.78	0.72	40.5	1.15	0.45	38.8	60.9
1-hour absorption period									
50 per cent dextrose	8	182–242	17.9	7.9	44.5	10.9	4.9	45.0	558.0
47.5 per cent sucrose	14	158–257	21.1	5.7	26.8	13.8	3.7	26.6	724.0
Corn syrup	11	176–296	9.8	4.3	43.6	6.5	2.9	44.7	352.0
2-hour absorption period									
50 per cent dextrose	9	162–250	40.1	8.0	20.0	25.4	5.0	19.8	1336.0
47.5 per cent sucrose	7	195–278	44.3	8.4	19.0	29.5	5.2	17.5	1542.0
Corn syrup	9	170–293	22.5	7.4	32.9	14.7	4.9	33.4	782.0
3-hour absorption period									
50 per cent dextrose	29	162–300	43.9	13.3	30.4	28.0	8.8	31.5	1557.0
47.5 per cent sucrose	27	137–384	63.2	20.0	31.8	45.8	9.9	21.6	2222.0
Corn syrup	15	228–300	34.7	8.8	25.4	24.7	6.3	25.4	1140.0
50 per cent dextrose and levulose	19	182–268	65.8	15.6	23.8	43.0	9.6	21.6	2617.0

The coefficient of variability per unit of surface area in the control animals was about 2 per cent lower than on the basis of body weight, but this difference is probably insignificant due to the extremely narrow range of weights of the animals in the series, and consequently no deduction as to whether basal liver glycogen bears a closer relationship to body weight or surface area can be drawn from the data in this table. It is evident, however, that liver glycogen is reduced to a very low level which, although not very constant from a statistical standpoint (coefficient of variability being 39 per cent), is nevertheless adequate for work where animal variation is a factor and where a difference to be expected will be great enough to show the trend of the reaction.

One hour after the administration of the three sugars studied, the data show no significant difference between the rates of glycogen formation from dextrose and sucrose (also fig. 2). The corn syrup, however, exhibits a rate of glycogen formation during the first hour which, on the average, is 50 per cent lower than that of dextrose and sucrose.

After 2 hours' absorption there is still little significant difference between the rates of glycogen formation in the liver from dextrose and from sucrose, the average difference between the two being much less than the standard deviation. The difference between these two sugars and corn syrup, as manifested during the first hour of absorption, still holds during the second hour, and the relation to the other sugars remains about the same.

After 3 hours of absorption there appears an appreciable difference in the rate of glycogenesis from dextrose and sucrose (table 5, fig. 3). The differences are clearly shown both on the basis of surface area and on the basis of body weight. Using average figures for comparison on the basis of weight, it may be stated that the rate of glycogenesis from sucrose after 3 hours of absorption is about 44 per cent greater than that of dextrose. Considering standard deviations, the difference is more significant on the basis of surface area, where the percentage increase is 60 per cent. The

glycogen content of the liver of those animals to which corn syrup was administered after this interval is, on the average, approximately 20 per cent lower than that of dextrose and 40 per cent lower than that of sucrose.

Since sucrose had been demonstrated to form more liver glycogen than dextrose after a 3-hour interval, it was important to determine whether the fructose part of the sucrose molecule had been responsible for the additional liver glycogen laid down by sucrose. In a separate series of animals a 50 per cent sugar solution containing dextrose and fructose in the

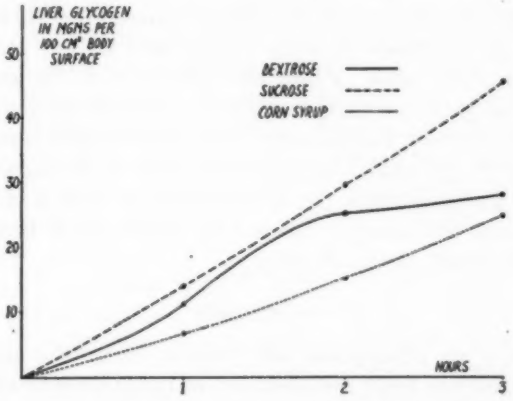

Fig. 3 Average glycogen content of livers at end of 1, 2 and 3 hours.

proportion in which they occur in the sucrose molecule was prepared and fed for a 3-hour absorption interval. The values obtained for liver glycogenesis after this period are quite similar to those obtained with sucrose. The rate of glycogen formation from this mixture of carbohydrates varies from that with sucrose by only a small fraction of the standard deviation.

In the 1- and 2-hour experiments, the coefficients of variability on the basis of surface area and body weight are almost identical as would be expected, since surface area has been calculated as a power function of the weight multiplied by

a constant. Also, from such a narrow range of weights of the animals used in these first two absorbing hours, a very narrow range of surface necessarily results (Murlin, '21). The series of animals used in the experiments for the 3-hour absorption period exhibit a wider range of weight variations, however, than those in other groups, the range being from 137 gm. to 384 gm., and for this reason the question of whether the glycogen content of the liver is more intimately related to body weight or to surface area can be briefly considered with respect to this group. The coefficients of variability on the two bases are approximately the same in the dextrose and corn syrup groups, but in the sucrose group the values are 32 per cent on the basis of body weight and 22 per cent on the basis of surface area. From the difference in the coefficients of variability under these conditions, one might feel inclined to deduce a closer relationship between glycogen and surface area, but the general variation and scatter of the values are much too great to allow the formulation of such a law. Liver glycogenesis obviously is related to other causal factors than the requirement for total energy.

DISCUSSION

The rate of absorption of glucose from the alimentary tract of rats has been reinvestigated, large groups of animals being used. The rate of absorption was not constant over a period of 3 hours as claimed by Cori and his associates. Rather the rate decreased with time in conformance with our former experiments and the results of various other workers cited under a review of the literature. The absorption coefficients obtained by different workers vary markedly for the 1-, 2- and 3-hour periods. For example, the coefficients obtained by Cori ('25) are lower than those obtained by Cori et al. ('29). In general, the same may be said for the results obtained by Pierce, Osgood and Polansky ('29) as compared with those presented in this paper. Cori et al. ('29) do not state whether their rats had been fasted 24 or 48 hours, but if for the former, higher coefficients would be expected as,

according to Cori ('31), prolonged starvation decreases the rate of absorption. Series C of the present study does not confirm throughout the idea that 48 hours' fasting causes a lessened absorption rate (compare 2- and 3-hour periods).

With animals carefully selected with regard to age and weight, large variations were noted in the amounts of sugar absorbed and, therefore, in the absorption coefficients. One set of data may be presented to illustrate this. Three rats weighing approximately 140 gm. were fed 1100 mg. of glucose. The absorption coefficients were 294, 213 and 232. Again, three rats weighing 145, 146 and 142 gm., respectively, were fed 1495, 1495 and 950 mg. of glucose. The absorption coefficients were 228, 240 and 469. We reaffirm our former statement that with adult rats, there is no constant relationship between the body weight and absorption, even when the amounts fed are constant. Moreover, the larger amounts fed often produce less absorption than the smaller amounts, particularly in the first hour (table 2). Coefficients of variation run as high as 47 per cent. They diminish with longer periods.

Rates of absorption do not determine specifically the rates of liver glycogenesis. It has been seen, for example, that corn syrup, in spite of its complex nature, is digested and absorbed, at an average rate, equal to that of cane sugar (table 4). Indeed, at the second hour it exceeds both sucrose and dextrose slightly (fig. 3). The rate of glycogenesis, however, following the administration of this mixture on a dextrose-equivalent basis with the other sugars, is consistently much less than that of sucrose and is farthest behind dextrose at the very point where its absorption overtakes that sugar. It approximates the rate of glycogenesis for dextrose at the third hour. It must be borne in mind that absorption rates and glycogenesis rates were determined on the same animals, the liver having been excised and dropped in hot caustic alkali, just preceding the ligation and removal of the alimentary tract. The values obtained indicate that the process depends more upon chemical configuration of the carbohydrate

involved, together with its effect upon the carbohydrate regulating mechanism of the organism itself, than on absolute absorption. The reverse relationship holds for sucrose, which having a slower absorption rate than dextrose at 2 and 3 hours, nevertheless formed slightly more liver glycogen than did dextrose at 2 hours and 44 per cent more at 3 hours.

Old experiments by Külz (1876) on rabbits by a method essentially the same as that which has become known in recent years as the Cori method, except that absorption was not determined, proved that fructose and sucrose are better producers of glycogen in the liver when fed in equivalent amounts than is glucose. His results were confirmed immediately by Frerichs (1876). The interval between feeding and death of the experimental animals is given as '4 to 5 hours.' The rabbits had been previously fasted for '6 full days.' Lusk (1892) showed that the formation of glycogen from fructose injected subcutaneously into rabbits took place at a more rapid rate than that from glucose, thus indicating that the difference obtained in favor of this sugar in older experiments was not dependent upon absorption rate. Cori ('26) confirmed this in rats which had been fasted 48 hours by showing that, whereas absorption of d-glucose took place twice as rapidly as fructose, the formation of glycogen from the latter was more than twice that from the former. The present experiments advance our knowledge a step further in showing that, whereas the absorption of a simple hexose takes place more rapidly than that of a double sugar, the rate of glycogen formation from the latter is more rapid. This conclusion that glycogen formation does not depend upon absorption rate is further reinforced by the feeding of a mixture of fructose and glucose. There can be no doubt that the excellence of sucrose as glycogen former is explained by the fructose fraction.

Glycogen values have also been computed throughout on the basis of liver weights, and, in general, these averages show the same tendencies as those computed on the basis of body weight and surface area. Less value in interpretation

can be attached to them, however, since the variation from animal to animal is much greater on this basis. It has also been found that the relationships between liver weight and body weight as well as those between total glycogen content and liver weight are exceedingly inconsistent.

CONCLUSIONS

1. The absorption of glucose from the gastro-intestinal tract of the rat does not take place at a constant rate, but falls during a 3-hour absorption period. The same statement, in general, holds true for sucrose and corn syrup.

2. The total amount of glucose absorbed and the amount absorbed per 100 gm. body weight per hour varied within wide limits and with adult rats the variations were not dependent upon body weight. Degree of filling of the alimentary lumen is believed to play an important role.

3. Although absorption per square centimeter of surface area varied markedly, the results indicate a slightly closer relationship between absorption and body surface than between absorption and body weight.

4. There seems to be some dependence of the percentage of glucose absorbed during 1-, 2- and 3-hour periods upon the amount of glucose remaining unabsorbed in the alimentary tract, although the results in different series are not uniform.

5. The rate of glycogenesis in the liver from dextrose and sucrose is approximately the same during the first and second hours after administration of the sugars, but during the third hour the rate of glycogenesis from dextrose falls off and sucrose forms approximately 44 per cent more liver glycogen than does dextrose. This effect is due to the presence of fructose in the sucrose molecule.

6. The rate of glycogen formation from corn syrup is lower than that of dextrose and sucrose, and proceeds at a fairly constant rate during the three hourly periods of absorption.

It is our pleasure to thank Dr. John R. Murlin for the advice and guidance given us during the period of this investigation.

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FAT FORMATION FROM SUCROSE AND GLUCOSE

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In the preceding paper (Feyder and Pierce, '35) it was shown that of the two carbohydrates d-glucose (dextrose) and sucrose, the latter is by far the better producer of glycogen and that this superiority is due to the fructose part of the sucrose molecule. The condition for glycogen retention in the body is, in general, identical with the condition for fat formation; that is to say, a supply of energy producing nutrients in excess of energy needs. It has often been surmised, but never proved, that glycogen is an antecedent step in the formation of fat (especially v. Wendt, '23; Hoffmann and Wertheimer, '27; Schoenen, '32). A little more light on this question would be obtained if we knew that, calorie for calorie, sucrose can produce fat more readily than glucose. Such a fact would indicate strongly, either, 1) that glycogen by reduction can be changed to fat, or, 2) that the keto hexose can be changed to fat more readily than the aldehyde hexose. In any case the outcome of such a comparison would have practical value.

Rubner (cited by Lusk, '28) found that starch, cane sugar and grape sugar are needed in the following equivalent amounts to replace 100 gm. fat in the metabolism of a fasting dog; starch 232 gm., cane sugar 234 gm., grape sugar 256 gm. But in the production of fat in domestic animals, according to the observations of Kellner ('05), starch is

[1] The writer wishes to express appreciation to the Corn Industries Research Foundation for the fellowship which supported this work.

superior to cane sugar in the proportion of 248 to 188 (i.e., grams fat from 1000 gm. carbohydrate). In other words, while cane sugar is equal to starch as direct fuel, in laying down a reserve of fuel (fat), it is only three-fourths as effective. So far as we have been able to find from a very thorough search of the literature, neither sucrose nor fructose has been compared directly with glucose as a source of fat.

v. Wendt ('23) argues that the keto sugar should not be so effective as the aldehyde, because only 3 C atoms would be available for synthesis, whereas, according to the equation of Magnus-Levy, modified by Lusk ('15 and '28) which pictures the transformation by way of methyl glyoxal, acetaldehyde and aldol condensation, either 4 or 6 C atoms would be available. v. Wendt, however, apparently was not aware of the older evidence for the superiority of fructose as glycogen former. It is certain that sufficient evidence is not at hand on which to predict with certainty the transformation of either hexose to fat on the basis of stereochemistry. The direct evidence for the superiority of fructose as glycogen former in the rat, rabbit and dog is conclusive. It would seem that the paired-feeding method accompanied by analysis of the entire body (of rats) should establish the order of merit of the two sugars for fat formation.

EXPERIMENTAL

A group of eleven control animals taken from a colony of selected inbred Wistar stock a short interval after weaning was placed on Purina dog chow diet for a period of 7 days. At the end of this period food was withdrawn for 24 hours, the animals were killed and the bodies analyzed by a method which will be described later (p. 462). The animals to be paired and fed were selected at weaning from litter mates whose mothers had been on the same stock diet. Paired mates of the same sex and approximately the same weight (table 1) were placed in separate cages with raised wire bottoms. Special food cups so constructed as to minimize spilling and wastage were used and the weights and food consumption

were recorded daily. The amount of food placed in the cups of each pair of animals daily was determined by the smaller amount eaten by one of the pair on the preceding day. Total food consumption was thus kept nearly equal day by day and was completely equalized once a week (Mitchell and Beadles, '30).

The diets fed to the two members of each pair differed only in the carbohydrate component, one of each pair receiving ordinary commercial sucrose and the other pure dextrose from corn.[2] The following represents the composition of the diets:[3]

Carbohydrate (sucrose or dextrose)	68
Casein (commercial)	18
Yeast (dried, Fleischmann's brewer's)	10
Salt mixture (Osborne and Mendel)	4
Cod liver oil, daily 3 drops	
	100

In the sucrose diet allowance was made for the fact that 95 parts sucrose produce 100 parts invert sugar upon intestinal hydrolysis, so that actually only 0.95 gm. of sucrose was ingested for each 1.0 gm. of dextrose.

In order to eliminate any possible difference in the caloric value of the two diets, which might of itself be responsible for any difference in growth or fat formation in each pair, duplicate samples of each diet were dried to constant weight and burned in the adiabatic bomb calorimeter. The dextrose diet contained 3.773 and 3.788 Cal. and the sucrose diet 3.782 and 3.779 Cal. per gram of dry food.

After the experimental animals had been on these diets for a period of 15 to 29 weeks, seven of the nine pairs were successively fasted for a period of 24 hours and the entire bodies were analyzed (p. 466).

During the course of the paired feeding on the sugar diets described above, certain symptoms appeared in various of the animals on or about the tenth week which resembled to some

[2] Supplied by Corn Industries Research Foundation.

[3] Thanks are due Dr. R. G. Daggs for suggesting this diet.

extent those described by Burr and Burr ('20) as evidence of the fat deficiency disease. The most important symptom seen was a marked scaling of the distal 3 to 4 cm. of the tail which in no case progressed to hemorrhage or necrosis, as is characteristic of the extreme fat deficiency. In some cases there appeared a temporary eczema on the hind feet without redness or swelling. There was no tendency to lose hair or for sores to appear on the skin. There was no evidence of hematuria or albuminuria. In pairs 1 to 4 (table 1) the scaly tail condition developed in all eight animals at the end of the tenth week, and was approximately identical both in severity and extent in the dextrose and sucrose rats. The condition improved spontaneously in pairs 1, 2 and 3, but never completely disappeared. The tail condition was most marked in the two members of pair 4, and was followed in about 4 weeks by a plateau in the growth curves of both animals. An extra supplement of 6 drops of cod liver oil daily fed to this pair equally was followed by rapid improvement of the tail condition and a resumption of growth, the sucrose animal responding slightly more rapidly.

In the second group of pairs carried simultaneously (viz., pairs 6, 7, 8, 9 and 10) the scaly tail condition occurred in only two-thirds of the entire group. The scaliness was of less severity than that which occurred in the first four pairs and involved only the distal 2 to 3 cm. of the tail. It appeared at the end of the seventh week in the sucrose animals and during the tenth week in the glucose animals. There was no interference with growth and the condition improved spontaneously in all animals affected, but never completely disappeared.

The diets fed contained no fat in the form of lard or butter, but no attempt had been made to produce a completely fat-free diet by extraction of the casein, yeast and cod liver oil as directed by Burr and Burr.

Results of paired feeding

In table 1 are shown in summary the results of feeding nine pairs of rats on the diets containing 68 per cent of sucrose and dextrose. In the column headed 'gain' it is apparent that in every pair the sucrose animal gained weight much faster than the dextrose rat in spite of the equality of food intake in grams and in calories. If the outcome was able to occur with equal probability in either of the two ways, that is, either the dextrose or sucrose rat might show the greater gain, then in a random selection of nine trials the frequency of its occurrence in one way would be given by the expansion of the binomial distribution $(1/2 + 1/2)^9$ (Fisher, '28). This means that its occurrence nine times in either way would result from chance, i.e., from equal effect of the two sugars, only once in 512 trials.

In the final column of table 1 are given the number of weeks each rat surpassed its mate. When weekly gains were equal, each rat was credited with 0.5 of a week. Of the 180 weekly comparisons 110.5 favored the sucrose rat and 69.5 favored the dextrose rat. The ideal outcome, if the sucrose did not affect the gain in weight more than the dextrose, would be 90 weeks favoring each sugar. The deviation from this in favor of sucrose is (110.5–90 =) 20.5. The probability of this occurrence as a mere matter of chance is found by comparing the figure 20.5 with the standard deviation of the frequency of distribution of 180 events each of which may result with equal probability. Fisher's ('28) formula for this standard deviation is—

$$\sqrt{0.5 \times 0.5 \times 180} = 6.71$$

The deviation of 20.5 from the expected outcome being 3.1 times the standard deviation of a chance distribution, it is quite clear that the former is not a matter of chance. On both methods of calculation of the odds, therefore, sucrose is shown to have been responsible for the greater increase in weight.

The analyses

It would not be proper to call this greater increase in weight 'better growth' without knowing the composition of the increase. Analyses of the entire body therefore were made,

TABLE 1

Paired feeding experiment

PAIR	SUGAR FED	SEX	INITIAL WEIGHT	FINAL WEIGHT	GAIN	TOTAL FOOD	WEEKS FED	NUMBER WEEKS IN WHICH EACH RAT SURPASSED ITS MATE
			gm.	*gm.*	*gm.*	*gm.*		
1	Dextrose	M	48	218	170	1853	22	10
	Sucrose	M	59	254	195	1853	22	12
2	Dextrose	M	66	267	201	2012	22	7.5
	Sucrose	M	62	312	250	2008	22	14.5
3	Dextrose	F	53	152	99	1990	29	11
	Sucrose	F	57	185	128	1963	29	18
4	Dextrose	F	71	212	141	1927	29	14
	Sucrose	F	66	230	164	1825	29	15
6	Dextrose	M	53	234	181	1186	16	8
	Sucrose	M	51	248	197	1193	16	8
7	Dextrose	M	66	240	174	1162	16	6.5
	Sucrose	M	63	256	193	1162	16	9.5
8	Dextrose	M	61	231	170	1180	16	5.5
	Sucrose	M	58	261	203	1180	16	10.5
9	Dextrose	F	80	172	92	998	14	3.5
	Sucrose	F	53	210	157	978	14	10.5
10	Dextrose	M	84	235	151	1235	16	3.5
	Sucrose	M	88	291	203	1232	16	12.5

1) of the control group of eleven animals selected from the same stock of weanlings as the paired rats, and 2) of all the pairs which survived in good health to the end of the feeding experiment, for protein (N), glycogen and fat. The method was as follows: The animals were killed by a blow on the

head, quickly cut into three portions, and dropped into a boiling solution of 30 per cent KOH. Solution of the tissues was complete in 15 minutes. The vessel contents were transferred to a 500 cc. volumetric flask and made up to volume after cooling to room temperature. Aliquot portions were then pipetted out for determination of nitrogen by the macro-Kjeldahl method, for determination of glycogen by the method of Good, Kramer and Somogyi ('33) and for determination of fat by the saponification method suggested by Leathes and Raper ('25) and modified by Dr. R. G. Sinclair,[4] of the Department of Biochemistry and Pharmacology. Fifty cubic centimeters of the partially saponified solution are taken and after addition of 10 cc. of absolute alcohol heating is continued for 3 hours under a reflux condenser. The KOH then is neutralized with HCl, diluted with 95 per cent alcohol to 40 per cent of the total volume and extracted with petroleum ether. After washing with water, separation in a separatory funnel and evaporation of the ether, the amount of fat is determined directly by weighing.

The composition of the control animals is given in table 2. Nitrogen is seen to be the least variable constituent, the standard deviation being only 0.083 gm. per 100 gm. body weight and the coefficient of variation only 2.9 per cent. The glycogen as milligrams per 100 gm. body weight and fat as grams per 100 gm. are about equally variable, the coefficients being 19.2 and 20.1 per cent, respectively. The method obviously is not suitable for a complete accounting of the body constituents. Preformed sugar, for example, probably is largely oxidized in the hot alkali, and ashing is out of the question. Carbohydrate in the diet, however, in so far as it had not been oxidized, might be expected to influence in a cumulative way only the three classes of organic constituents given. The protein retention and, therefore, protoplasmic growth might be affected by the sparing effect. Glycogen would be the resultant of two principal factors, aside from

[4] Thanks are due Doctor Sinclair for helpful suggestions regarding the use of this method.

molecular configuration, viz., food intake and activity, but being to so large an extent a form of temporary storage the amount reported would be most affected by recent activity, i.e., since the last time of eating. Fat would most nearly represent the difference between energy supply and consumption over a long period of time, but if one form of carbohydrate were more readily converted to fat, or if a more suitable residue were left after the energy requirements were met from one of the hexoses than from the other, the differ-

TABLE 2

Body composition of control animals at beginning of diet period

RAT NO.	SEX	WEIGHT AFTER 24-HOUR FAST	TOTAL N	GRAMS N PER 100 GM. BODY WEIGHT	TOTAL GLYCOGEN	MILLIGRAMS GLYCOGEN PER 100 GM. BODY WEIGHT	TOTAL FAT	GRAMS FAT PER 100 GM. BODY WEIGHT
		gm.	*gm.*		*mg.*		*gm.*	
1	F	63			64.6	102.6	3.35	5.32
2	M	75	2.11	2.82	81.0	107.9	2.61	3.48
3	F	69	1.92	2.78	81.0	117.6	2.57	3.72
4	M	107	2.80	2.62	145.8	136.0	4.06	3.79
5	M	104	2.81	2.71	84.7	82.5	5.05	4.86
6	M	72	2.02	2.80	100.1	140.4	2.18	3.03
7	M	76	2.23	2.94	69.9	92.0	3.01	3.95
8	M	74	2.08	2.81	100.1	136.4	2.54	3.42
9	M	68	1.93	2.84	88.6	130.2	1.99	2.93
10	M	68	1.93	2.84	87.9	129.2	2.23	3.28
11	M	71	2.06	2.90	97.5	137.2	2.29	3.23
Average				2.81		110.1		3.73
Standard deviation				0.083		21.2		0.75
Coefficient variability				2.9%		19.2%		20.1%

ence might be expected to show in the comparison of controls with the survivors.

From this point of view it is disappointing that the variation in percentage of fat contained in the controls was so large. Probably a preliminary period of equalized food intake, say, of a couple of weeks after weaning, instead of a period of ad libitum feeding, would have improved this result.

Of the nine pairs of rats fed equal quantities for as long as 14 weeks (table 1), pair 4 developed serious signs of fat

deficiency, as explained on page 459, and one member of pair 9 died of pneumonia at 14 weeks. The remaining seven pairs were analyzed (table 3). Again it is seen that the protein (N per 100 gm. body weight) is the least variable constituent, and in the dextrose and sucrose rats averages almost exactly the same. The agreement in standard deviations and coefficients of variation is very satisfactory. There is only a slight increase in percentage of nitrogen over the young controls. The average difference in glycogen content is 4.6 mg. per 100 gm. body weight in favor of the dextrose animal. This is too small in comparison with the standard deviations to be significant. The average difference in fat content is 4.0 gm. per 100 gm. body weight, in favor of the sucrose group. Standard deviations and coefficients of variation on so small a number of animals are not reliably representative and therefore are not of special interest except to indicate whether the series analyzed at the end was approximately as concordant as the series of controls analyzed at the beginning. The sucrose animals were nearly as concordant, but the dextrose animals were not. The increase in percentage of fat over the control is note worthy—3.7 per cent to 13.3 and 17.3 per cent for the two groups.

On the basis of total fat content, the sucrose animal in every pair exceeded the dextrose animal by from 4 gm. (no. 8) to 31 gm. (no. 2). Without reference to the amount of difference, this result in the entire seven trials could have been obtained as a matter of chance only once in 128 trials. On the percentage basis, only six of the seven pairs showed a greater amount of fat for the sucrose animal, but even this result could occur as a matter of chance only once in sixty-eight times, since so far as known every other food factor was equalized. It, therefore, is quite clear that the greater percentage of fat produced in the sucrose rats as well as the greater gain in weight was caused by some peculiarity of the sucrose molecule. It is equally clear that of the organic constituents determined only fat was thus favored. On the basis of the superiority of fructose as glycogen former, it is prob-

TABLE 3

Body composition of rats at end of diet period

PAIR	SUGAR FED	SEX	WEIGHT AFTER 24-HOUR FAST	GAIN IN WEIGHT	TOTAL N	GRAMS N PER 100 GM. BODY WEIGHT	TOTAL GLYCOGEN	MILLIGRAMS GLYCOGEN PER 100 GM. BODY WEIGHT	TOTAL FAT	GRAMS FAT PER 100 GM. BODY WEIGHT
			gm.	*gm.*	*gm.*		*mg.*		*gm.*	
1	Dextrose	M	207	170	7.20	3.48	74.8	36.1	18.2	8.8
	Sucrose	M	239	195	6.79	2.84	99.7	41.7	34.3	14.4
2	Dextrose	M	252	201	7.85	3.12	117.6	46.8	26.4	10.5
	Sucrose	M	296	250	8.74	2.96	131.6	44.5	57.3	19.4
3	Dextrose	F	148	99	4.61	3.11	72.5	48.9	6.1	4.1
	Sucrose	F	181	128	5.59	3.09	83.8	46.3	14.4	7.9
6	Dextrose	M	225	181	5.99	2.66	155.6	69.2	47.3	21.0
	Sucrose	M	237	197	6.93	2.92	120.6	51.0	59.9	25.2
7	Dextrose	M	228	174	6.37	2.79	198.5	87.9	25.1	11.0
	Sucrose	M	244	193	8.84	3.62	181.2	74.3	35.8	14.7
8	Dextrose	M	214	170	5.43	2.54	63.8	29.8	42.1	19.6
	Sucrose	M	243	203	6.38	2.62	65.6	27.1	46.1	19.0
10	Dextrose	M	220	151	5.52	2.54	69.4	31.6	40.6	18.4
	Sucrose	M	274	203	6.79	2.48	82.5	30.1	56.8	20.8
Average	Dextrose					2.89		49.63		13.34
	Sucrose					2.94		45.04		17.34
Standard deviation	Dextrose					0.32		19.0		5.88
	Sucrose					0.34		11.8		5.15
Coefficient variability	Dextrose					11.1%		38.4%		44.0%
	Sucrose					11.5%		26.2%		28.4%

able that the greater production of fat was due to this part of the sucrose molecule, and we have one more link in the chain of evidence that glycogen formation and fat formation are intimately related.

The greater fattening effect of sucrose cannot be the result of a lesser specific dynamic effect, for the investigations of Lusk ('15), Bürger ('21) and Wierzuchowski ('31) prove that fructose has a greater effect on heat production than glucose. Furthermore, Carpenter and Fox ('30) have shown that the higher R.Q.'s following ingestion of fructose than glucose very probably are due to more rapid transformation into fat, and Carpenter and Lee ('33) have proved that no reduction of alveolar CO_2 is involved, even though more lactic acid may be produced (see Wierzuchowski for lactic acid production after intravenous injection). Finally, there was no sign of greater activity in the dextrose group, although this was not measured.

SUMMARY

1. By the paired feeding method, as improved by Mitchell and Beadles, rats were placed on diets identical in all respects, except that one member of each pair received dextrose and the other sucrose, as the chief source of energy. Bomb calorimetric determinations proved that the two diets were equal in heat value.

2. Without exception (nine pairs), the sucrose animal gained weight more rapidly than the dextrose animal. This result could not have happened by chance oftener than once in 512 trials. On the basis of the number of weeks each rat surpassed its pair mate the total result was 110.5 for the sucrose rat against 69.5 for the dextrose rat, the deviation from a chance frequency distribution being 3.1 times the standard deviation of such a distribution of 180 events.

3. Control young animals and seven pairs of the survivors after 16 to 29 weeks of feeding were analyzed for nitrogen (protein), glycogen and total fat. The results were not quite so concordant in the mature animals as in the controls, but showed clearly that the difference in the weight gains was due principally to fat.

4. It is concluded, therefore, that sucrose has a considerably greater fattening effect in the rat than dextrose.

The writer wishes to express his appreciation to Dr. John R. Murlin for his advice and assistance throughout this investigation.

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THE EFFECTS OF A DEFICIENCY OF IODINE AND VITAMIN A ON THE THYROID GLAND OF THE ALBINO RAT [1]

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TWO PLATES (NINE FIGURES)

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INTRODUCTION

This investigation, dealing with the influence of dietary factors on the thyroid gland of the albino rat, was undertaken because recent direct experimental evidence challenges the generally accepted theory that endemic goiter is due to lack of iodine. The deficiency of iodine has been stated by Marine ('09), Hayden, Wenner and Rucker ('22), Tanabe ('25) and Drennan, Malcolm and Cox ('31) to cause: 1) a definite hypertrophy of the thyroid; by Knappenburg ('19), Hellwig ('31), Jackson and P'An ('32), 2) no hypertrophy; by Wegelin ('29) and Hellwig ('31), 3) atrophy; and by Marine ('09) and Thompson ('33), 4) hypertrophy followed by atrophy. Dietary defects other than iodine deficiency have also been stated to cause hypertrophy of the thyroid. McCarrison ('31) has attributed hypertrophy to vitamin A deficiency, and Hellwig ('31) has attributed it to an excess of calcium. On the other hand, Drennan, Malcolm and Cox ('31) obtained no hypertrophy in rats fed diets deficient in vitamin A or D, and Sampson and Korenchevsky ('32) obtained similar negative results in male rats fed on a diet deficient in vitamin A only. It has also been stated that iodine deficiency accom-

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panied by some other dietary defect causes pronounced hypertrophy of the thyroid gland of rats. Sampson and Korenchevsky ('32) obtained such hypertrophy with diets deficient in vitamin A and iodine; Fischer ('33) with diets deficient in vitamin B and iodine; and Tanabe ('25), Krause and Monroe ('30), Hellwig ('31), Thompson ('32 and '33), and Levine, Remington and Von Kolnitz ('33) with iodine deficient diets containing an excess of calcium.

Analysis of the procedure employed in the above investigations reveals that conflicting results may have been due to differences in one or more of the following factors: a) the composition of the diets which, in all except those used by Jackson and P'An and Sampson and Korenchevsky, were mixed diets of which the exact composition was unknown; b) the strain of the rats which were either Norwegian or albino; c) the initial age, as a difference in susceptibility of the thyroid gland of man to iodine deficiency at different ages is known to exist according to Marine ('09); d) the duration of the experiment, as Marine ('09) and Thompson ('33) have pointed out that an initial hypertrophy may be followed by atrophy of the thyroid; e) the sex of the rats, as it is commonly known that there is a higher incidence of goiter among women than among men in regions where goiter is endemic.

MATERIAL AND METHODS

Every attempt was made in the present investigation to eliminate all factors other than those under consideration. To ensure this the following precautions were taken: litters of albino rats were obtained from the same colony; all animals were subjected to uniform dietary procedure previous to the period of experimentation; effects of deficiencies were studied on both sexes; litter mates of the same sex and approximately the same weight were selected as controls for experimental animals; highly purified diets were used, adequate in all respects, or deficient only in the factor or factors under investigation; distilled drinking water was supplied; and experiments were run for varying lengths of time, ranging from 2 weeks to 4 months.

Litters of rats, obtained from the Department of Physiological Chemistry of Yale University, were so prepared during the period of lactation as to limit and render more uniform their reserves of vitamin A. This was done because some of the rats were to be fed a vitamin A free diet, and it was desirable to use the same preliminary dietary procedure with all animals. The resultant low reserves could be exhausted, in rats placed on vitamin A deficient diets, within a few weeks of weaning.

The age of the animals at the beginning of the experiment ranged from 22 to 28 days, with the exception of one litter which was 33 days old. The rats in each litter were weighed; those of comparable sex and weight were paired as control and experimental animals; and each one was placed in a separate cage. Seventy-seven individuals, including males and females constituting thirteen litters, were divided into the following groups designated according to their diets: adequate diet (C), iodine deficient diet (—I),[3] vitamin A deficient (—A), and doubly deficient (—A—I). Food and water were supplied ad libitum. The composition of the diets is indicated in table 1.[4]

Careful observations of the animals were made daily throughout the experiments, body weights and food consumption were recorded twice a week, indications of vitamin A deficiency were noted, and daily weights of the rats recorded when signs of deficiency first appeared. The animals on the

[3] The iodine 'deficient' diet contained 16 to 17 gamma per kilogram or 16 to 17 parts per billion of iodine, according to analyses made in duplicate by Prof. Roe E. Remington. The expense of the analysis was defrayed by a special grant from Smith College.

[4] The casein used in the control diet was obtained from the Casein Company of America, that in the experimental diets from the Harris Laboratories; the salt mixture was made according to the Osborne and Mendel formula ('13), the iodine being omitted from the iodine-free salt mixture; the irradiated yeast was obtained from the Standard Brands Company; the carotene, generously provided by the companies concerned, was fed in the form of S.M.A. 'caritol' to the first two litters, as a solution of Mead Johnson crystalline carotene in maize oil to the remaining litters. Both solutions of carotene were effective in supplying adequate amounts of vitamin A. The Harris casein and the crystalline carotene were used because of their greater purity and possible freedom from iodine.

iodine deficient diet, with their paired controls, were killed at intervals of 2 to 16 weeks. Animals on the vitamin A deficient and doubly deficient diets were killed when a loss in body weight or signs of xerophthalmia appeared, except those of litters VII, XI, XII and XIII. In these the rats were killed at a set time in order to observe the effects of the deficiencies over definite periods. Indications of vitamin A deficiency appeared at different times in different litters, ranging from 3 to 5 weeks, except in one litter in which no external sign of

TABLE 1

The composition of diets

(C)		(—I)	
	gm.		*gm.*
Vitamin A free casein	180	Vitamin A free casein	180
Starch (corn)	530	Starch (corn)	530
'Crisco'	240	'Crisco'	240
Complete salt mixture	50	Iodine-free salt mixture	50
Yeast, irradiated	180	Yeast, irradiated	180
Carotene (2 drops of 0.3 per cent daily)		Carotene (2 drops of 0.3 per cent daily)	
(—A)		(—A—I)	
	gm.		*gm.*
Vitamin A free casein	180	Vitamin A free casein	180
Starch (corn)	530	Starch (corn)	530
'Crisco'	240	'Crisco'	240
Complete salt mixture	50	Iodine-free salt mixture	50
Yeast, irradiated	180	Yeast, irradiated	180

deficiency had appeared at the end of 2 months when the animals were killed. Professor Anderson, from whom the rats were obtained, reported similar irregularity in the exhaustion of vitamin A reserves with other litters prepared in the same manner as those used by us.

The animals were killed by illuminating gas. At autopsy the body weight was recorded, the amount of body fat noted but not weighed, the condition of the lungs and the vascularity of the thyroid, as indicated by color, noted carefully. All endocrine and male reproductive organs were weighed after remaining in Allen's modification of Bouin's fixative for 24

hours, according to the technic proposed by Korenchevsky ('30). The fixed material was subsequently used for histological study. Sections of the thyroid glands, 7 μ in thickness, were stained with hematoxylin and eosin, by Bensley's method ('16), and with Mallory's triple stain. Excellent results were obtained with the latter, after first treating the sections with solutions of mercuric chloride, iodine and sodium thiosulphate, as recommended by Carleton ('26). These triple stained sections, especially, were used for most of the histological study, and the other sections compared with them.

RESULTS

1. General conditions

The final body weights of the animals on the control diet, although greater than normal according to Donaldson's figures ('24) were, with one exception, below the average and in some instances below the minimum for rats of the same age from the Yale colony (Smith and Bing, '28). A comparison of final body weights demonstrates that consistent differences existed only between controls and those rats on vitamin A deficient diets in which obvious symptoms of the deficiency occurred. In the latter, loss of appetite was accompanied by fall in weight, xerophthalmia, and in a few instances by the loss of muscular coordination described by Aberle ('34). Additional evidence of vitamin A deficiency, noted at autopsy, was depletion of fat and in males degeneration of the reproductive organs. In one control, no. 41, which failed to consume the food provided, loss of weight ensued and evidence of malnutrition was noted at autopsy. In none of the rats was there any indication of respiratory or other infection. No consistent differences in vascularity of the thyroid gland, as indicated by color, were found in the animals on the deficient diets as compared with their litter mate controls.

2. Correlation of thyroid weight with body weight

The actual thyroid weight, in the majority of individuals on all diets, varied directly with the body weight, as is indicated

TABLE 2

Protocol of male rats

DIET	LITTER NUMBER	RAT NUMBER	INITIAL AGE	TIME ON DIET	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	THYROID WEIGHT	THYROID WEIGHT PER 100 GM. BODY WEIGHT
			days	*days*	*gm.*	*gm.*	*mg.*	*mg.*
	VII	43	25	14	52	70	8.8	12.5
	VIII	54	27	14	41	76	11.4	15.0
	III	17	23	21	42	92	12.4	13.5
	VI	41	23	61	46	97	15.4	15.8
	VIII	75	23	20	48	105	15.6	14.8
(C)	II	11	22	37	36	108	14.6	13.5
	IV	23	23	24	46	108	19.8	18.3
	X	62	34	14	92	123	15.4	12.5
	XI	69	25	28	42	127	24.6	19.3
	IX	56	28	24	60	140	22.2	15.8
	VI	39	23	92	42	152	18.8	12.3
	V	28	23	59	52	224	36.4	16.2
	XIII	77	23	20	46	67	12.0	17.9
	III	16	23	21	40	75	11.2	14.9
	VIII	55	27	14	44	79	14.2	17.9
	XIII	76	23	20	33	87	11.8	13.5
	X	63	34	14	86	110	16.0	14.5
	XI	67	25	28	36	115	19.6	17.0
(—I)	IV	24	23	24	42	120	13.4	11.1
	XI	68	25	28	38	124	17.8	14.3
	XI	66	25	28	30	127	18.0	14.1
	II	9	22	37	38	128	13.2	10.3
	VI	37	23	61	41	138	16.8	12.1
	VI	35	23	92	42	188	19.4	10.3
	V	29	23	59	52	219	28.6	13.0
	V	32	23	59	48	220	33.6	15.3
	III	15	23	21	44	42	5.6	13.3
	IV	22	23	24	42	64	10.8	16.8
	VII	45	25	14	44	80	12.0	15.0
(—A)	VII	48	25	14	52	82	10.0	12.2
	VII	47	25	14	52	84	8.6	10.2
	II	13	22	37	38	103	13.0	12.6
	II	12	22	37	42	130	14.4	11.0
	V	30	23	59	51	174	18.6	10.6
	III	14	23	21	44	70	10.8	15.4
	IV	21	23	24	46	72	13.4	18.6
	IX	57	28	24	67	84	14.0	16.6
(—I)	IX	59	28	24	62	95	16.2	17.0
(—A)	IX	58	28	24	66	117	14.0	11.9
	II	10	22	37	38	122	15.8	12.9
	V	31	23	59	48	148	16.4	11.1

in the protocols of the males and females as given in tables 2 and 3. Such a correlation has been shown to exist in rats by Donaldson ('24) and in rats and guinea pigs by Friedman and Loeb ('34).

TABLE 3

Protocol of female rats

DIET	LITTER NUMBER	RAT NUMBER	INITIAL AGE	TIME ON DIET	INITIAL BODY WEIGHT	FINAL BODY WEIGHT	THYROID WEIGHT	THYROID WEIGHT PER 100 GM. BODY WEIGHT
			days	*days*	*gm.*	*gm.*	*mg.*	*mg.*
	VI	40	23	47	42	63	9.4	14.9
	XII	74	26	21	27	75	10.0	13.3
	XI	72	25	14	40	80	8.8	11.0
	VIII	50	27	21	42	88	12.6	14.3
	IX	60	28	14	57	104	12.8	12.3
(C)	I	5	28	28	55	108	12.2	11.3
	X	64	34	14	90	118	15.2	12.8
	IV	27	23	92	34	120	18.4	15.3
	V	33	23	61	46	154	23.8	15.4
	III	18	23	119	44	155	23.0	14.8
	VII	42	25	37	50	162	19.4	11.9
	VI	38	23	47	34	66	10.6	16.0
	VIII	51	27	21	40	73	13.8	18.9
	VI	36	23	47	42	84	12.2	14.5
	IV	25	23	92	42	88	14.4	16.3
(—I)	IX	61	28	14	57	94	15.4	16.3
	I	1	28	28	66	134	17.2	12.8
	IV	26	23	92	42	140	18.6	13.2
	X	65	34	14	90	146	19.4	13.3
	III	20	23	119	44	152	20.2	13.2
	III	19	23	119	46	164	22.8	13.9
	XI	70	25	14	36	46	7.2	15.6
	VIII	52	27	21	39	53	7.6	14.3
	XII	73	26	21	40	53	9.8	18.5
	XI	71	25	14	40	60	9.0	15.0
(—A)	VII	46	25	37	50	68	9.6	14.1
	I	7	28	28	58	72	11.0	15.2
	VII	49	25	37	51	76	13.6	17.9
	VII	44	25	37	44	116	17.0	14.6
	I	8	28	28	68	122	18.4	15.0
	VIII	53	27	21	37	49	7.2	14.7
(—I)	I	4	28	28	67	104	13.0	12.5
(—A)	I	3	28	28	74	111	18.6	16.7

3. Comparative weights of thyroid glands per 100 gm. of body weight

Table 4 includes the weights referred to below.

(*—I*) *Diet.* In male rats, an iodine deficient diet for from 14 to 21 days produced hypertrophy of the thyroid gland as compared with the glands of litter mate controls. This diet over a period of 25 to 92 days caused slight atrophy. In females hypertrophy was more pronounced than in males and persisted up to the forty-seventh day on the deficient diet. Atrophy occurred in females fed the deficient diet for periods of 47 to 119 days.

(*—A*) *Diet.* In male rats on the vitamin A deficient diet the thyroid glands were, with one exception, consistently smaller than in their litter mate controls, the contrast being most pronounced at the end of the experimental period of 59 days. In females, on the contrary, the thyroid glands were enlarged in the rats on the vitamin A deficient diet for periods of 14 to 37 days as compared with those on the control diet. The onset of symptoms of vitamin A deficiency prevented the continuance of females on this diet for longer periods, which might have resulted in atrophy of the thyroid.

(*—A—I*) *Diet.* In males, on a diet deficient in both vitamin A and in iodine, the weights of the thyroids were, with one exception, slightly greater than in their litter mate controls at the end of 24 days. In male rats fed the doubly deficient diet for 37 days, there was slight atrophy, and in those on the diet for 59 days more pronounced atrophy. In the females on the deficient diet for 21 to 28 days there was slight hypertrophy. Onset of the symptoms of vitamin A deficiency precluded the investigation of effects of more prolonged feeding with the doubly deficient diet.

4. Effects of the diets on the histological structure of the thyroid

(*C*) *Diet.* The histology of the thyroid of the rats on the control diet, that is, of the normal gland, is characterized by

TABLE 4

Differences in weight of the thyroid glands per 100 gm. of body weight

DIETS	MALES					FEMALES				
	Litter number	Control rat number	Experiment rat number	Time on diet	Difference in thyroid weight	Litter number	Control rat number	Experiment rat number	Time on diet	Difference in thyroid weight
				days	*mg.*				*days*	*mg.*
	VIII	54	55	14	+ 2.9	IX	60	61	14	+ 4.0
	X	62	63	14	+ 2.0	X	64	65	14	+ 0.5
	XIII	75	76	20	— 1.3					
			77	20	+ 3.1					
	III	17	16	21	+ 1.4	VIII	50	51	21	+ 4.6
	IV	23	24	24	— 7.2					
	XI	69	66	28	— 5.2	I	5	1	28	+ 1.5
(–I)			67	28	— 2.3					
versus			68	28	— 5.0					
(C)	II	11	9	37	— 3.2					
						VI	40	38	47	+ 1.1
								36	47	— 0.4
	V	28	29	59	— 3.2					
			32	59	— 0.9					
	VI	41	37	61	— 3.7	V	33	34	61	— 2.8
	VI	39	35	92	— 2.0	IV	27	26	92	— 2.1
						III	18	19	119	— 0.9
								20	119	— 1.6
	VII	43	45	14	+ 2.5	XI	72	71	14	+ 4.0
			47	14	— 2.3			70	14	+ 4.6
			48	14	— 0.3					
	III	17	15	21	— 0.2	XII	74	73	21	+ 5.2
(–A)						VIII	50	52	21	+ 0.0
versus	IV	23	22	24	— 1.5					
(C)						I	5	7	28	+ 3.9
								8	28	+ 3.7
	II	11	12	37	— 2.5	VII	42	44	37	+ 2.7
			13	37	— 0.9			46	37	+ 2.2
								49	37	+ 6.0
	V	28	30	59	— 5.6					
	III	17	14	21	+ 1.9	VIII	50	53	21	+ 0.4
	IX	56	57	24	+ 0.8					
			58	24	— 3.9					
(–A–I)			59	24	+ 1.2					
versus	IV	23	21	24	+ 0.3					
(C)						I	5	3	28	+ 5.4
								4	28	+ 1.2
	II	11	10	37	— 0.6					
	V	28	31	59	— 5.1					

marked variation, typical of the normal human gland (Marine, '09, and Reinhoff, '30); and of the duck thyroid (Severinghaus, '33). Variations occur in the structure of the thyroid in different individuals, different parts of the same gland, and different areas of the same follicle. The height of the cells lining the follicles varies from low to high cuboidal (fig. 1); mitotic figures and colloid droplets occur in some and not in other follicular and interfollicular epithelial cells; vesicles vary in size, shape and in the amount, distribution, vacuolization and staining capacity of the colloid within them. The peripheral area of the colloid in the vesicles and the intracellular colloid invariably took the basic stain in the Mallory and the Bensley technics, which indicates its lability and the fact that it is newly formed or resorbed colloid (Severinghaus, '33). The remainder of the colloid in most cases took the acid stain, but in some follicles the entire colloid mass took the basic stain. The process of transfer of colloid is evidently transcellular in view of the location of similar colloid droplets in vacuoles in the following areas: within and at the edge of the colloid mass in the vesicles, at the free border, and within the epithelial cells lining the vesicles (figs. 2 and 3). Transcellular method of resorption has been described in amphibia (Grant, '31); and in the duck, sheep, guinea pig and monkey (Severinghaus, '33). New follicles form within the follicular and interfollicular epithelium by the method described for sheep by Severinghaus ('33), and also by a constriction of large colloid masses in the follicles, and the subsequent enclosure of the two resultant masses by the reorientation of some of the epithelial cells. There is no evident relation between the occurrence of mitotic figures and new follicle formation.

(*—I*) *Diet.* In rats on the iodine deficient diet slight hyperplasia is evident at the end of 28 days and occurred in both males and females on the diet for 56 days. It is characterized by the height and particularly by the dome-shaped free edge of the epithelial cells lining the follicles (figs. 4 and 7). This hyperplasia is succeeded by atrophy in males fed this

deficient diet for 84 days, and in females on the diet for 122 days. The characteristics of the atrophic thyroid gland (fig. 9) are the large size of the vesicles throughout, the flattened epithelial cells lining the vesicles, the retraction of the colloid from the free edge of the cells, staining indicating the resorption of colloid, and the greater amount of connective tissue as compared with that in the thyroid glands of litter mate controls.

(—*A*) *Diet.* The thyroids of both male and female rats on this deficient diet show two effects: 1) hyperplasia of epithelial cells similar to that observed in rats on an iodine deficient diet for a short period, and, 2) the degeneration of the epithelial cells which seems to be a specific effect of vitamin A deficiency (fig. 5).

(—*A*—*I*) *Diet.* In male and female rats on this deficient diet for 21 to 28 days, two effects appear, namely, hyperplasia and degeneration. There is no consistent difference in the colloid content of the vesicles of the deficient and the control rats nor in the number of mitoses, but there is more follicle formation. In a male rat on the doubly deficient diet for 56 days, atrophy occurred, accompanied by degeneration of the membrane and of the cytoplasm of the epithelial cells lining the vesicles (fig. 6).

When the thyroid gland of rats on the experimental diets are compared with those of litter mate controls, it becomes evident that changes in percentage weight preceded the hyperplastic, and later the atrophic, changes in the structure of the glandular epithelium. It is possible that early modifications in the weight of the thyroid are due to initial changes in its vascularity, and in the consistency of the colloid within it, which precede changes in the function and structure of the follicular epithelium.

DISCUSSION

The body weights of the rats fed the control diets are lower than those given by Smith and Bing ('28) for rats of the same age from the same colony, due probably to the fact that the

preliminary dietary procedure restricted the vitamin A reserves and the resultant growth of these rats. The thyroid weights of the majority of these control rats correspond more closely to Donaldson's figures for the thyroid weights of animals of comparable age, than of those of comparable body weight. This appears to indicate that age is the factor determining thyroid weight, but that the latter is modified by body weight, as in rat no. 41. No consistent differences exist in the thyroid weights of males and females on the control diet.

The variation in the histological structure of the thyroids of rats on the normal diet is evidence of the lability of the normal gland, and favors the theory that activity of the gland is cyclic, periods of active secretion alternating with the periods of rest or of resorption of colloid, and activity of one part accompanying inactivity of another region of the same gland. The presence of mitotic figures and the prevalence of newly formed follicles indicate growth of additional thyroid epithelium in control and deficient rats. The location and the character of the colloid-containing vacuoles give evidence of the transcellular resorption of the colloid from the vesicles.

(—*I*) *Diet.* Iodine deficiency in males and females for brief periods induces hypertrophy, and over longer periods causes atrophy of the thyroid. This is indicated by the differences in weight of the thyroid glands per 100 gm. of body weight in rats on the iodine deficient diet as compared with their litter mate controls, and by the comparative histological structure of the thyroid glands of the experimental and the control rats. These observations confirm Marine's statement ('09) that iodine deficiency over brief periods may induce compensatory hypertrophy, which may with time be followed by an exhaustion atrophy. Neither hypertrophy nor atrophy occurs in rats on control diets containing iodine. The most pronounced atrophy on this iodine deficient diet occurs in rats on the diet for the longest periods, and confirms the statement of Hellwig ('31) that "the severity (of the atrophy) . . . . is in direct proportion to the length of time the feeding experiments are continued." The fact that the diet used contains

iodine to the extent of 16 to 17 gamma per kilogram may account for the failure to obtain more marked hyperplasia in this investigation. The results indicate that the hypertrophy occurs only during the first few weeks after weaning, that it is transitory, and that it is followed by atrophy. Results reported by Thompson ('33) indicate that in rats the hypertrophy is more pronounced and persistent when an excess of calcium is added to an iodine-deficient diet.

(—*A*) *Diet.* Vitamin A deficiency alone led to different results in these experiments in females as compared with male rats. In the former it caused hypertrophy as indicated by comparison of thyroid weight per 100 gm. of body weight with that of litter mate controls, and as indicated by the protrusion of the free edge of epithelial cells into the cavity of the follicles. A possible explanation of the hyperplasia is that the vitamin A deficiency first alters the normal metabolism of the epithelial cells of the thyroid gland and prevents the normal absorption and utilization of iodine; and is thus a dietetic factor which causes a 'relative' deficiency of iodine (Marine, '28; and Thompson, '33). Histological examination reveals that degeneration of some of the epithelial cells occurs simultaneously with hyperplasia of others in the same follicle (fig. 5). In males the vitamin A deficiency causes atrophy, even in individuals on the diet for a very brief period. A deficiency of longer duration might produce atrophy in females. In the males the degenerating effect, so clearly evident on histological study, apparently predominates, as is indicated by the comparative weights of the thyroids per 100 gm. of body weight.

(—*A*—*I*) *Diet.* The effects of the iodine and the vitamin A deficient diet on the thyroid may be viewed in the light of the effects of the single deficiencies. In males for a period of 3 weeks, and in females for one of 4 weeks, the double deficiency produces slight hypertrophy as indicated by weight; but associated with it is some degeneration revealed by histological examination. In males over a period of 37 to 59 days atrophy, associated with degeneration, occurs as revealed by

weight and histological appearance. It is probable that atrophy in females would also have occurred if they could have been maintained for a longer period on the doubly deficient diet.

SUMMARY

1. Iodine deficiency in the diet produces an initial hypertrophy of the thyroid gland in both males and females. This persists for a longer time in females. It is followed in both sexes by atrophy.

2. Vitamin A deficiency produces definite hypertrophy in the female, during the limited period of experimentation, but consistent atrophy in the male.

3. A deficiency of iodine and vitamin A in the diet produces an initial hypertrophy in males and females. In males atrophy follows with continued deficiency.

4. Enlargement of the thyroid gland, induced experimentally, is more pronounced and persists longer in females than in males.

5. A study of the normal rat thyroid indicates that: a) Great variability exists in the histological structure of the normal gland, which may be regarded as evidence of a cyclic secretion of the glandular epithelium; b) colloid release in the normal rat thyroid is evidently a transcellular process.

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PLATE 1

EXPLANATION OF FIGURES

Camera lucida drawings, × 1500. Colloid represented in solid black. Details added free hand from oil immersion study.

1 Portion of a single follicle of thyroid of normal rat, no. 40, female, on adequate diet 47 days. Note variability in height of epithelial cells, ranging from cuboidal to high columnar. Also size of follicle.

2 A colloid droplet outside of the vacuole in the colloid mass, passing toward the epithelium.

3 A colloid droplet passing through the membrane of the free edge of the epithelial cell.

4 Follicle and epithelium of adjacent follicle of rat no. 38, female, on iodine deficient diet for 47 days. Note the hyperplasia of the epithelial cells; the 'dome' shape of the individual cell.

5 Follicle of rat no. 13, on vitamin A deficient diet for 37 days. Note degenerating epithelial cells at bottom and right of follicle. Also the 'dome' shape of the free edge of cells at the left. Colloid droplets present in epithelial cells.

6 Single follicle of rat no. 21, on iodine and vitamin A free diet for 24 days. Note degeneration of epithelial cells.

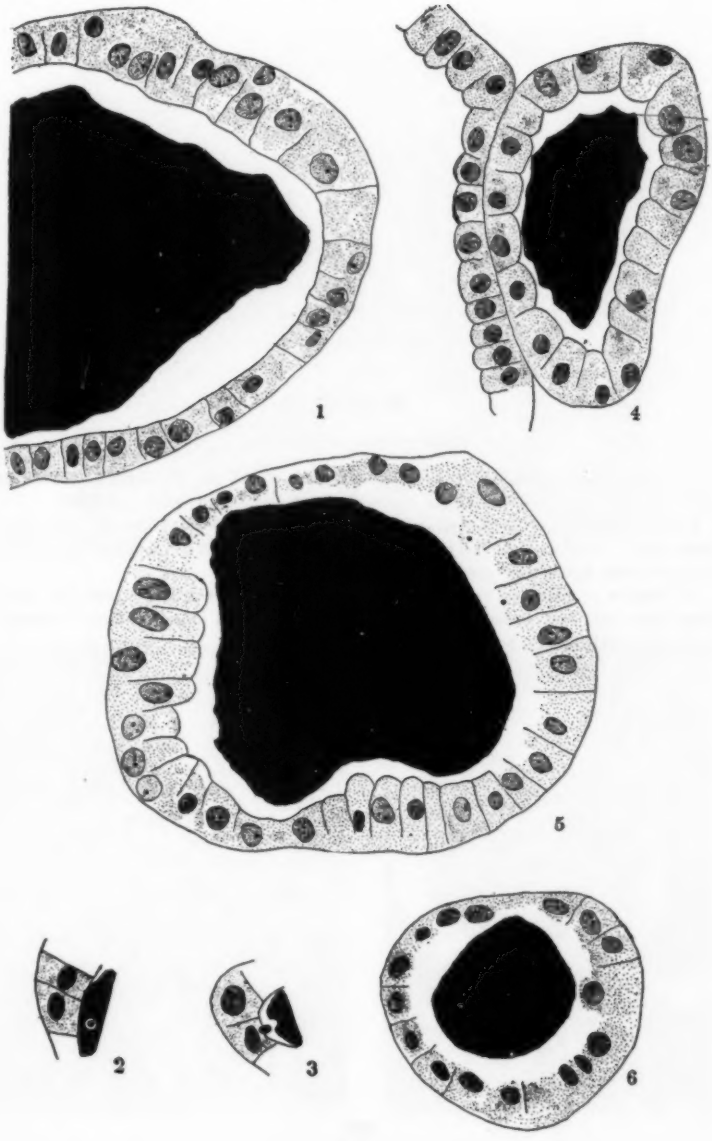
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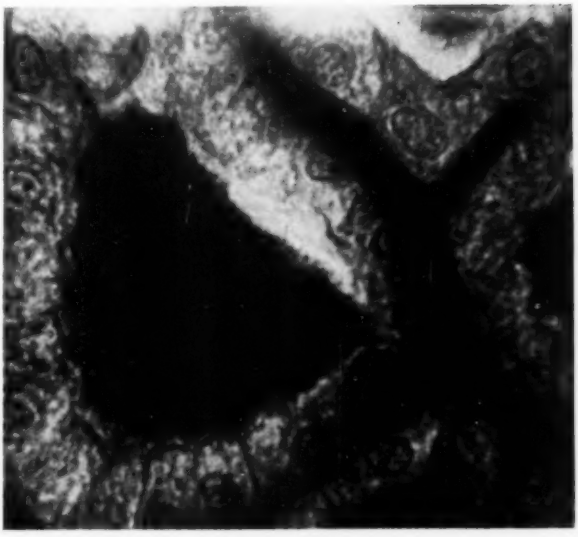
PLATE 2

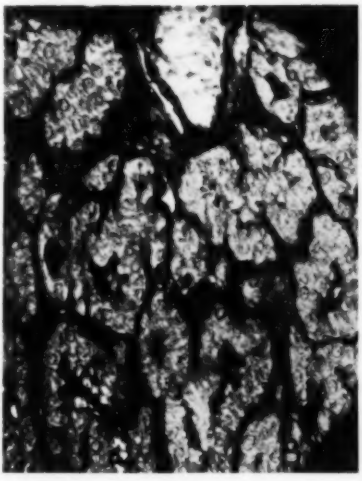
EXPLANATION OF FIGURES

7 Follicle and epithelia of adjacent follicles of rat no. 38, female, on iodine deficient diet for 47 days. Note hyperplasia of epithelial cells. × 1400.

8 Portion of gland of control rat no. 18, female, on adequate diet for 119 days. Note many small vesicles, height of epithelial cells and small amount of colloid. Compare with figure 9 of same magnification. × 300.

9 Portion of gland of rat no. 19, female, on iodine deficient diet for 119 days. Note large size of follicles, flattened epithelial cells, and stored colloid, retracted from the epithelium. × 300.





UTILIZATION OF GELATIN, CASEIN AND ZEIN BY ADULT RATS [1]

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The relative utilization of gelatin, zein and casein was studied by McCollum ('12) in experiments on the pig. At that time relatively little was known of the mineral and vitamin requirements of animals. The present investigation was undertaken to determine the relative value of these same proteins in the nutrition of the adult rat in the light of the present knowledge of accessory needs. Since McCollum's aim was to determine the influence of a given protein in repairing waste due to endogenous catabolism, he wished to avoid feeding above the endogenous level, and took the urinary nitrogen alone as an index of the nitrogen to be fed. He regarded the fecal nitrogen as accidental in character and independent of the essential tissue metabolism, but of use in determining the thoroughness of absorption.

It is generally recognized that the biological value of a protein for maintenance is underestimated if it is fed above the endogenous level, since a portion of it would then be used for energy requirements. In the case of young growing rats, Mitchell ('23–'24 b) found that corn proteins fed at a 5 per cent level had a biological value of 72, while a 10 per cent level

[1] Part of the data reported in this paper are taken from a thesis presented by Inez D. Mason in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, 1933. The remainder were secured with the aid of a fellowship granted by the Graduate School of the same university. The original data are on file in the Division of Biochemistry, University of Minnesota.

which permitted some growth gave a value of only 60. Whether or not the values are constant for varying levels below that required for maintenance is still a disputed question. Mitchell and Hamilton ('29) consider it logical to suppose that dietary amino acids are oxidized in the tissue ". . . . in proportion to their relative concentration in the intracellular fluids with reference to the other organic nutrients . . . . and also in proportion to their relative oxidizability as compared with glucose, fats, lipoids, etc." Hence the biological value of a protein may be expected to increase as its percentage in the diet decreases.

Widely varying results have been given for the utilization of gelatin when fed at approximately the endogenous level. McCollum ('12), with pigs, found a utilization of 50 to 60 per cent, while Terroine, Fleuret and Stricker ('23), also with pigs, gave nitrogen balance data from which a biological value of 33 may be calculated. Mitchell ('23–'24 b), with rats, got results ranging from 0 to 44.

Casein was given a value of 70 by Thomas ('09), 71 by Mitchell ('23–'24 b) and 76 by Fixsen and Jackson ('32). Rajzmann ('33) found a biological value of 77, 78 and 81 in experiments with the rat, the pig and the rabbit, respectively.

Like gelatin, zein has given variable results. Calculations from Henriques' ('09) nitrogen balance data give a value of 30, while McCollum ('12) records a value of 80.

In this work we used Mitchell's ('23–'24 a) method for determining biological values.

EXPERIMENTAL

Preparations

Since the food intake decreases after a few days on a 'nitrogen free' diet, a study was made of palatability. The inclusion of sugar and a change of fat helped the appetite. The diet chosen contained tapioca dextrin 60 per cent, sugar 19 per cent, Osborne and Mendel's ('19) salt mixture 4 per cent, agar 2 per cent, butter fat 13 per cent, and cod liver oil 2 per cent. This was alternated with a similar diet containing

slightly more lard in the place of butter fat and cod liver oil, and with slightly less sugar. These diets contained 0.22 mg. nitrogen per gram food. Allowance was made for the difference in the energy contents of the two diets in providing for the calculated energy requirements of the different animals.

In order to provide a vitamin supplement low in nitrogen, yeast was extracted as follows:

Five hundred grams of Fleischmann's whole dry yeast were extracted with 50 per cent alcohol three times, the total amount of alcohol used being 5 liters. In each extraction the mixture was heated with constant stirring, to the boiling point, then filtered through a Büchner funnel. The combined filtrates were added to 220 gm. of dextrin, evaporated, ground and

TABLE 1

Nitrogen and water content of casein, zein and gelatin

	WATER	NITROGEN
	per cent	*per cent*
Casein	4.85	15.73
Gelatin	11.11	17.58
Zein	4.37	15.94

made into tablets. Each tablet contained the extract from 500 mg. of yeast and weighed 300 mg. The nitrogen content of these tablets prepared from various yeast extracts was 6.2, 8.2 and 5.5 mg. per tablet, respectively.

The casein was prepared by Palmer and Kennedy ('27), and the zein by Mason and Palmer ('34). A commercial sample of gelatin was used.

Analyses

Samples of these three proteins were analyzed for moisture by drying for 5 hours in a vacuum oven at 100°C. and for total nitrogen by the micro Kjeldahl method. The results are shown in table 1.

All analyses for total nitrogen were made with the Cavett ('31) apparatus, using the regular Kjeldahl method.

The feces were prepared for analysis by air drying, removing loose hair, and grinding. In some cases a large amount of hair was evident after the grinding. This was shaken to the top and the greater portion removed with forceps. Before weighing samples for analysis, the ground feces were again shaken and the top layer containing some hair was brushed aside so that the samples were taken from the lower layer without hair.

In determining the nitrogen distribution in the proteins a few changes were made in Cavett's ('32) modification of the

TABLE 2

Nitrogen distribution of casein, gelatin, zein and the alcohol soluble fecal extract after feeding zein as determined by the Van Slyke analysis. Expressed in per cent of total nitrogen

	CASEIN	GELATIN	ZEIN	ALCOHOLIC FECAL EXTRACT
	per cent	*per cent*	*per cent*	*per cent*
Ammonia N	10.30	3.86	19.68	21.39
Humin N	1.89	0.52	1.28	2.23
Arginine N	7.82	15.17	3.78	2.77
Cystine N	0.26	0.21	0.17	0.23
Histidine N	7.54	4.33	3.26	2.00
Lysine N	9.80	6.85	1.24	2.38
Total N of bases	25.42	26.56	8.45	7.38
Filtrate from bases:				
Amino N	57.71	53.06	62.40	64.24
Non-amino N	5.00	17.12	7.06	4.63
Total recovery	100.32	101.12	98.87	99.87

Van Slyke method. The hydrolysis, distillation of hydrochloric acid and determination of ammonia nitrogen and total humin nitrogen were made in the Patton ('32) Kjeldahl-Claissen flask. Twice the quantity of material was taken throughout, the phosphotungstate separation was made by filtration and the histidine fraction obtained by calculation. The results are shown in table 2.

Experimental technic

Adult male rats weighing 350 to 500 gm. were selected at random from various laboratory strains.

The metabolism cages were made by inverting in a rack a battery of bottle tops serving as funnels. In each funnel was placed a round cage with a removable bottom. A water bottle was inserted at the side, and from the top hung a covered feed cup of the McCollum type. A copper wire screen was placed under the cage bottom in the funnel to retain the feces, while underneath the funnel was a beaker to receive the urine. The copper mesh, funnel and beaker were disinfected with an alcoholic solution of thymol to prevent decomposition of the urine, and as an added precaution against loss of ammonia 5 cc. of a 3 per cent sulfuric acid solution were placed in each beaker.

The plan of the experiment was to use a 3-day preliminary period during which the basal ration was fed from the hanging feed cups, but no collections were made. This was followed by a 2-day period in the metabolism cages. At the end of this period urine and feces were collected and analyzed. The animals were put into other cages and given in addition to the previous diet about 0.4 gm. of the protein to be used in the following period. After analyzing the urine, the amount of the desired protein which contained the same amount of nitrogen was calculated. During each of the three succeeding days each rat was given the amount of nitrogen as protein which had been excreted in urine daily by the same individual during the previous period. At the end of 3 days, collection of urine and feces was again made, and protein feeding was discontinued. After a day's interval the rats were returned to the metabolism cages for a final 2-day 'nitrogen free' feeding period, after which collections and analyses were again made. A yeast extract tablet soaked in cod liver oil was given each rat daily. The animals were kept in individual cages at all times during the experiment.

The accuracy of the collection of urine was tested by trickling a known volume of urine over each of two cage bottoms at intervals during a 3-day period. Thirty cubic centimeters of urine were used in each cage, and at the end of the period the collection was made by thoroughly washing the cage bot-

tom, funnel and copper wire netting, using distilled water and a small brush. About 200 cc. of water were used in each case. This was made up to 250 cc., filtered, and 5 cc. duplicates taken for analysis. Analysis of the original specimen showed a loss of only about 3 per cent in collection.

Since the food intake progressively decreases on a diet very low in nitrogen, it seemed desirable to make the preliminary period as short as possible in the hope of securing an adequate energy intake throughout the experiment. In order to determine the length of time required to bring the urinary nitrogen to the endogenous level, experiments were conducted on six rats fed on a 'nitrogen free' diet. The urine was collected and analyzed daily. Only a very slight decrease was found after the third day, so a 3-day preliminary period was chosen.

It seemed desirable to feed the protein separately, since each rat was given a different amount, and it was impossible to predict from day to day just how much of the ration would be eaten. The problem was to feed the protein in such a form that it would be palatable and not easily scattered, and so that any which was thrown out could be recovered for analysis. With gelatin these conditions were met by adding 1 gm. of sucrose to each day's protein ration and then making it into a stiff gel with water. Casein and zein presented difficulties as they were easily scattered and were unpalatable in their highly purified condition. These proteins were incorporated in a sweetened agar gel. The casein was mixed with 10 cc. of a 1 per cent agar sol containing 1 gm. of sucrose and allowed to gel. Zein did not mix well with the sol, so it was beaten into the gel with a piece of copper wire. Fresh protein ration was prepared each day and at the end of 3 days all material left in the dishes as well as any which was scattered was collected and analyzed for nitrogen.

Although the basal diet contained 2 per cent of agar, the increase of this substance during the protein feeding period raised the question as to whether the increase of indigestible material in the diet altered the amount of fecal nitrogen eliminated. Mendel and Fine ('12) found that the addition of agar

plus bone ash to the diet of a dog increased the nitrogen output. Mitchell ('23–'24 a) thought that the addition of indigestible matter in the form of filter paper was not of great importance in determining biological values, although he found an appreciable increase in metabolic fecal nitrogen.

In order to test the effect of this additional agar, seven rats were carried through the regular experimental periods, except that protein was not given. They were given the regular amount of extra agar as a sweetened gel during the interval and the 3-day collection period. There was no appreciable change in the amount of nitrogen eliminated per gram of food eaten.

Nitrogen balance data

Twenty nitrogen balance experiments were carried out with casein, eighteen with gelatin and twenty with zein. The relative utilization was calculated by the McCollum ('12) method in which the fecal nitrogen is not included. The results are given in table 3, the average values being 74 for casein, 23 for gelatin and 57 for zein.

The nitrogen balance data were studied to determine significant correlations and possible errors inherent in the method.

The increase in urinary nitrogen during the growth of the rat was shown by Hatai ('05). In the present experiment only adult males were used, and since the differences in weight were partly due to fat, the question arose whether there would be a larger excretion of urinary nitrogen in the heavier animals. Table 4, no. 1 shows a highly significant correlation between the weight of the animal and its endogenous urinary nitrogen even with adult rats ranging in weight from 341 to 504 gm. The tests for significance are taken from the table of Wallace and Snedecor ('31).

The following formulae were used:

$$\sigma x = \frac{\sqrt{\Sigma x^2 - (\Sigma x)\,\overline{x}}}{\sqrt{n-1}} \qquad r = \frac{\Sigma xy - (\Sigma x)\,\overline{y}}{\dfrac{n-1}{\sigma x\, \sigma y}} \qquad t = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

During the experiments each animal lost weight. This loss was partly due to a decrease in the contents of the alimentary

canal. Several rats were weighed the day after the completion of the experiment having had stock food for 24 hours. The average gain was 11 gm. The other fraction was probably chiefly loss of fat. This was only gradually regained. In

TABLE 3

Percentage retention of casein, zein and gelatin calculated by McCollum's method

RAT NO.	CASEIN	ZEIN	GELATIN
11		60.7	20.1
13			23.5
14			23.1
16	76.0		22.9
17	78.8	69.0	31.8
17		74.3	23.3
20	67.6	51.5	32.8
27	84.6		35.5
27			16.7
29	73.9	50.9	
30			17.8
33	76.3	47.3	17.1
34			17.0
35	75.0	50.0	
36	82.7		
37	70.7		40.4
38			30.3
39	76.3	49.2	22.7
41	77.7		15.1
42	74.5	58.5	14.9
45	78.2		
50	80.3		
51		72.8	
52	77.0	51.9	
52		72.9	
55	64.0		
56	72.5	59.3	
59	71.7	64.0	
60	70.0	58.9	
61	60.1	47.8	16.1
61		57.1	
63		44.1	
64		46.6	
67		59.9	
Mean	74.4	57.3	23.4
Standard deviation	5.95	9.41	7.69

TABLE 4

Summary of nitrogen balance data

FACTORS CORRELATED	n	$\bar{x}$	$\bar{y}$	σx	σy	r	t
1. x: Weight of rat y: Endogenous urinary N	30	399.8	71.4	38.96	9.25	+ 0.482	2.91
2. x: Percentage retention gelatin N y: Percentage retention zein N	7	21.6	55.9	6.59	8.46	+ 0.213	0.486
3. x: Percentage retention gelatin N y: Percentage retention casein N	10	23.6	74.3	8.41	6.74	— 0.036	0.010
4. x: Percentage retention zein N y: Percentage retention casein N	12	56.4	72.9	7.34	5.06	+ 0.255	0.834
5. x: Mg. N in yeast and basal diet y: Percentage retention zein N	20	8.5	57.3	1.78	9.41	— 0.364	1.660
6. x: Mg. N in yeast and basal diet y: Percentage retention gelatin N	18	7.9	23.4	0.81	7.69	+ 0.268	1.111
7. x: Ingested zein N as percentage endogenous urinary N y: Percentage retention zein N	20	83.4	57.3	17.54	9.41	— 0.444	2.101
8. x: Ingested gelatin N as percentage endogenous urinary N y: Percentage retention gelatin N	18	95.2	23.4	23.41	7.69	— 0.477	2.168
9. x: Ingested casein N as percentage endogenous urinary N y: Percentage retention casein N	20	105.2	74.4	9.61	5.95	— 0.318	1.424
10. x: Percentage ingested zein y: Percentage retention zein N	20	4.24	57.3	1.98	9.41	— 0.344	1.555
11. x: Percentage ingested gelatin y: Percentage retention gelatin N	18	3.98	23.4	1.86	7.69	— 0.471	2.136
12. x: Percentage ingested casein y: Percentage retention casein N	20	5.52	74.4	1.58	5.95	— 0.198	0.859
13. x: Fecal zein N as percentage ingested N y: Urinary zein N as percentage resorbed N	20	20.7	55.1	8.77	15.74	+ 0.738	4.890
14. x. Fecal corn N as percentage ingested N y: Urinary corn N as percentage resorbed N (Mitchell)	21	6.0	40.1	3.15	6.54	+ 0.608	3.334
15. x: Fecal cocoa N as percentage ingested N y: Urinary cocoa N as percentage resorbed N (Mitchell)	13	60.0	59.6	7.92	13.32	+ 0.659	2.907
16. x: Efficiency index y: Fecal zein as percentage ingested N	22	1.71	27.8	0.44	8.26	— 0.251	0.116
17. x: Efficiency index y: Urinary zein N as percentage resorbed N	22	1.71	61.7	0.44	10.65	+ 0.029	0.132
18. x: Efficiency index y: Biological value of zein	22	1.71	38.3	0.44	10.64	— 0.030	0.133
19. x: Fecal zein N as percentage ingested N (rats of known efficiency) y: Urinary zein N as percentage resorbed N	22	27.8	61.7	8.26	10.65	+ 0.102	0.462

determining the endogenous urinary nitrogen of young rats, Mitchell ('23–'24 a) introduces corrections for changes in body weight during the experiment. In order to see whether this correction is necessary with large adult rats, twelve experiments were recalculated using corrections for body weight and compared with the uncorrected values. There was no appreciable difference in the figures.

The greatest difficulty of the experiment lay in securing adequate calorie intake and many animals were discarded temporarily because they ate so little. The surface area was calculated from the modified Meeh-Rubner formula as given by Lee ('29). This for male rats is $S = 12.62 \times W^{0.60}$, where S = body surface in square centimeters, and W = weight in grams. Hitchcock ('27) calculates that inactive mature rats require approximately 855 calories per square meter of body surface. The amount of food required was calculated and when the intake fell far below this amount, the rat was discarded. It seemed possible that with fat mature animals a somewhat lowered food intake for a brief period might not be of great importance, since the animal's supply of fat would furnish energy. One might expect that a lowered intake of energy during the protein feeding period would give a lowered retention of nitrogen, since part of the protein would probably be used for energy requirements. On the other hand, a lowered calorie intake during the 'nitrogen free' periods might be expected to give too high a percentage retention because of giving too high a value for the endogenous nitrogen. This would mean too low a value for the exogenous fraction and in consequence a high percentage retention.

In order to determine the significance of the calorie intake, a study was made of the individual cases. Of the fifty-eight experiments there were nineteen in which the energy intake fell below the theoretical requirements in one or both of the 'nitrogen free' periods, but was adequate during the protein feeding period. This showed no appreciable effect on the percentage retention of nitrogen. Similar findings are reported by Fixsen and Jackson ('32). There were only two

cases in which the intake was inadequate during the protein feeding period, but adequate during the other periods. In these two cases the values were distinctly below the average, though not so low as the lowest values in experiments in which the intake was adequate throughout. It was assumed, therefore, that no serious error was introduced into the average results due to inadequate food intake.

Since in some cases the same animals were used in experiments with more than one protein, it seemed desirable to know whether a rat which utilized one protein well would give similar results with another. Correlations were made between the percentage retention of the nitrogen of gelatin and zein, gelatin and casein, and zein and casein. Nos. 2, 3 and 4 in table 4 show no relation between the retention of the different proteins.

As the 'nitrogen free' diet and the vitamin supplement both contained a small amount of nitrogen, the question arose as to the possibility of a supplementary action between these forms of nitrogen and the protein studied. To test this the supplementary nitrogen ingested was correlated with the percentage retention of nitrogen in the case of each of the inadequate proteins. No relationship was found (table 4, nos. 5 and 6).

The constancy of the biological value of any protein for maintenance when fed in amount less than that required for nitrogen equilibrium is still a debated subject. Martin and Robison ('22) are inclined to the view that it is constant, while Mitchell ('23–'24 b) believes that the animal organism becomes more economical as less protein is supplied, so that higher values are obtained for smaller quantities ingested. He believes that the biological value is related to the percentage of proteins in the food, since a decreased calorie intake would increase the utilization of protein for energy.

In the present work each animal was given in the form of protein the amount of nitrogen which it had excreted in urine during the previous 'nitrogen free' period. Varying amounts of the offered protein were eaten, so we were able to correlate

the ingested nitrogen expressed as its percentage of the endogenous urinary nitrogen with the percentage of nitrogen retention. The average of the two 'nitrogen free' periods is used in the correlations. This explains an ingestion of over 100 per cent in no. 9 (table 4, nos. 7, 8 and 9). Similar calculations were made in which the ingested protein was expressed as percentage of the food intake (table 4, nos. 10, 11 and 12). These two sets of correlations were uniformly negative, two in the first set and one in the second being significant, thus indicating a higher retention when less nitrogen is ingested.

As a check on the digestion of the proteins several fecal analyses were made for nitrogen in the experiments with gelatin and with casein. The digestion of gelatin was found to be complete and that of casein nearly so. In eight cases the biological values were calculated by the method of Mitchell and the results showed no appreciable difference from those calculated by McCollum's retention method.

On the other hand, zein was not well digested, and the digestibility varied with different animals. For this reason nitrogen determinations were made on the feces of all zein fed animals and the biological values were calculated according to Mitchell's method. These values showed an extremely wide variation, ranging from 18 to 72 with a standard deviationof 15.86. In investigating this wide range of values a correlation was run between the exogenous fecal nitrogen expressed as the percentage of the nitrogen ingested and the exogenous urinary nitrogen expressed as the percentage of the nitrogen resorbed. The purpose of this was to determine whether there was any connection between the two types of wastage of nitrogen, i.e., that lost through lack of digestion and resorption and the further loss of part of the resorbed portion through failure to utilize it in metabolism. This gave a highly significant correlation, as shown in table 4, no. 13.

In a further study of this relationship the nitrogen balance data (Mitchell, Beadles and Keith, '26) where cocoa was fed, also Mitchell's ('23–'24 b) experiment in which corn was fed at the 10 per cent level were treated in the same manner. In

the case of cocoa, the correlation between the wastage of food nitrogen in the feces and its wastage in the urine was significant, and in the experiment with corn it was highly significant.

These correlations throw light on the wide range of biological values obtained when the animals vary widely in their ability to digest the protein. However, the fundamental explanation for this relationship remains to be determined.

Composition of undigested zein

Mitchell and Hamilton ('29) believe that the portion of the food protein which escapes digestion may differ markedly in its amino acid yield from the ingested food, since the different linkages in the molecule vary in resistance to digestive enzymes. According to this theory, most of the molecules may be partially broken down in the alimentary canal and the more resistant portion excreted. After feeding zein to rats, Osborne and Mendel ('14) were able to recover from 7.4 per cent to 39.5 per cent of the ingested nitrogen by extracting the feces with 80 per cent alcohol. This indicates that the zein residue is still alcohol soluble in spite of changes which may have taken place in its composition. If certain groups of little or no value in nutrition should be split off and resorbed first, most of this nitrogen would be excreted in the urine. In order to test this possibility, an alcoholic extract of the feces of zein fed rats was analyzed for nitrogen distribution.

After a preliminary period on a diet containing dextrin 54 per cent, sugar 10 per cent, salt mixture 4 per cent, agar 2 per cent, zein 15 per cent and lard 15 per cent, feces were collected from nine rats for 4 days. Samples were analyzed for total nitrogen. The metabolic nitrogen was calculated from the average of thirty previous experiments (1.91 mg. per gram food consumed). According to this calculation, 23 per cent of the food nitrogen was present in the feces. After ether extractions to remove the fat the feces were extracted with 92 per cent alcohol. The alcoholic extract contained 16.4 per cent of the ingested nitrogen, and 71 per cent of the calculated exogenous fecal nitrogen. Analysis of this extract is

given in table 2. The nitrogen distribution, not being markedly different from that of the ingested zein, gave no evidence in support of this theory.

Nitrogen balance experiments with rats of known efficiency index

It seemed possible that the individual variation in ability to digest zein might be related to the inherent food utilization efficiency of the animals. To test this, twenty-two nitrogen balance experiments were carried out as before, using male rats whose efficiency index had been determined during the 6-week period after attaining a weight of 60 gm. These animals were supplied by Palmer and Kennedy ('31) and were from inbred strains having at least nine generations of sib matings and which showed great uniformity in ability to digest synthetic diets. The efficiency indices[2] ranged from 1.13 to 2.44. These indices were correlated with the exogenous fecal nitrogen expressed as the percentage of ingested nitrogen, with the exogenous urinary nitrogen expressed as the percentage of resorbed nitrogen, and with the biological value. The exogenous fecal nitrogen expressed as the percentage of ingested nitrogen was also correlated with the exogenous urinary nitrogen expressed as the percentage of resorbed nitrogen. None of these correlations are significant (table 4, nos. 16, 17, 18 and 19).

We suggest that the insignificant correlation in the latter case may be explained by the use of the advanced inbred strains in contrast to the former case where random sampling of rats resulted in a highly significant correlation between the fecal and urinary wastage of the food.

SUMMARY

Nitrogen balance experiments were carried out with adult male rats, using casein, gelatin and zein. The percentage retention calculated by McCollum's ('12) method averaged 74 for casein, 23 for gelatin and 57 for zein.

[2] The efficiency index is the food required (in grams) per gram of gain per 100 gm. body weight.

Studies were made of the nitrogen balance data, first, to determine possible errors inherent in this type of experiment, and, second, to find significant correlations.

With the large adult male rats used it was found unnecessary to correct the urinary nitrogen for the loss in weight during the experiment.

A fall in calorie intake somewhat below the theoretical requirements during the 'nitrogen free' feeding period was of no apparent significance.

There was no apparent supplementary effect of the nitrogenous compounds of the yeast extract and the dextrin on the inadequate proteins used.

The percentage retention increased as less protein was ingested, even though it was never fed above the endogenous level.

When the same animals were used in experiments with more than one protein, there was no significant correlation between the individual utilization of the different proteins.

Gelatin and casein were so well digested that there was no significant difference between the percentage retention calculated by McCollum's ('12) method and the biological value calculated by Mitchell's ('23–'24 a) method. However, zein showed a poor and variable digestibility, the exogenous fecal nitrogen ranging from 4 to 36 per cent of the ingested zein. The biological values varied widely, from 18 to 72, the average being 45. This wide spread in results led to the discovery of a highly significant correlation between the exogenous fecal nitrogen expressed as the percentage of nitrogen ingested and the exogenous urinary nitrogen expressed as the percentage of nitrogen resorbed.

Further experiments with rats of known efficiency index for growth showed no relation between the inherent efficiency of the animal and the digestibility or utilization of the zein.

Analysis of undigested zein residues for nitrogen distribution furnished no explanation of the highly significant correlation between the food wastage in the feces and that in the urine.

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THE VITAMIN C REQUIREMENT OF THE GUINEA PIG

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ONE CHART

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This study consists of a determination of the amount of vitamin C required by guinea pigs ranging from 124 to 1024 gm. in body weight. As a criterion of vitamin deficiency, the changes in microscopic structure of the roots of the incisor teeth, first described by Zilva and Wells ('17–'19) and analyzed in detail by J. A. Höjer ('24) and A. Höjer ('26), have been employed. Three reasons for collecting these data may be given. The first is the establishment of the relationship, if any, between body size and vitamin C requirement. Although it has been shown by Goettsch and Key ('28) and confirmed by Eddy ('29), that a 300 gm. guinea pig needs about 3 cc. of orange or lemon juice daily for protection against the earliest signs of scurvy, namely, the tooth changes, there seems to be no information available as to the requirements of larger or smaller animals. Would one expect a young guinea pig of 100 gm. weight and an old one of 900 gm. to require 1 cc. and 9 cc. of lemon juice, respectively, or relatively more or less? A quantitative answer to this question, in the form of a mathematical equation, might shed light on the actual factors (e.g., body mass, age, growth, or metabolic

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[2] A part of the expense of this investigation was defrayed by the Research Fund of the Yale University School of Medicine.

rate) possibly concerned in the body's use of vitamin C, in a manner analogous to what has already been demonstrated by Cowgill and his collaborators ('32) with respect to vitamin B.

The second reason why such data might be of value resides in the fact that they may assist in the determination of the human requirement for vitamin C in the same way that animal studies have done in relation to vitamin B (Cowgill, '34). Direct measurements of the vitamin C needs of human beings are difficult to make. Göthlin ('31) and Falk, Gedda and Göthlin ('32–'33), using the strength of skin capillaries as a criterion, have reported that man probably requires from 0.7 to 1.0 cc. of orange juice daily per kilogram of body weight, or relatively about one-ninth as much as the guinea pig. Measurement of the requirements of different sized members of the latter species might aid in understanding why the human requirement should be relatively so much smaller.

A third, though less important, purpose in our study is a possible contribution to the convenience of workers who use biological methods for the vitamin C assay of foods. The knowledge now available as to the chemical nature of this dietary factor, and the rapidly developing methods for the determination of ascorbic acid, may shortly render obsolete the use of guinea pigs in assay of food materials. Nevertheless, as long as biological assays are still employed, a formula for relating vitamin C requirement of the body size in this species might make it possible to use animals of any weight; the investigator would then not be limited to the use of guinea pigs between 250 and 350 gm.

METHODS

The diet employed was Eddy's ('29) modification of the Sherman-La Mer scorbutic ration and consisted of the following ingredients:

	Per cent
Skim milk powder, heated in a current of air at 110°C. for 24 hours	30
Butter fat	9
Sodium chloride	1
Rolled oats and whole bran, mixed in equal parts by weight	59
Cod liver oil	1

Eighty guinea pigs, studied in groups of from six to ten at a time, gave the results reported in this paper. Usually three or four animals of the same sex and approximate size were housed in a large cage. The small animals were obtained from a local dealer, while the larger ones were purchased from Prof. H. L. Ibsen, of Kansas State College of Agriculture, who has used for genetic studies strains characterized by unusually large individuals. Groups of various sizes were selected more or less at random over a period of a year, so that possible seasonal differences were avoided. For example, a group ranging from 120 to 200 gm. in body weight was studied in January, and another of similar size in May; some animals of 700 to 800 gm. were observed in April, and others in October.

After a few days, during which the guinea pigs received oats, cabbage and scorbutic food mixture, ad libitum, they were restricted to the scorbutic diet alone, supplemented with individual daily doses of fresh decitrated lemon juice, prepared as directed by the Conference on Vitamin Standards of the League of Nations (Medical Research Council, '32). The juice was given by pipette, and little difficulty was experienced in training most of the animals to take it quantitatively without spilling. Water was supplied ad libitum in drinking fountains consisting of inverted bottles provided with glass tubes. After a period of subsistence on the scorbutic diet plus limited vitamin C, in a few instances 11 days, but usually 14 days, the guinea pigs were chloroformed and the lower jaws quickly dissected out and placed in 8 to 10 per cent formalin. A superficial examination of the joints was made in about thirty of the animals, but no gross scorbutic changes were observed. The abdominal cavities were opened to ascertain whether or not any of the females were pregnant. It was found that the larger animals (700 gm. or over) always contained a relatively greater deposit of fat around the kidneys and genital tracts. In nineteen cases, the adrenals were removed for silver nitrate staining by the technic described by Moore and Ray ('32).

The weights given are those of the animals just before the period of subsistence on the scorbutic diet. A certain degree of inaccuracy cannot be avoided here, inasmuch as the alimentary tracts of some animals may have contained more material than others; also, most of the smaller guinea pigs were growing rapidly during the 2 weeks' period, while some of the largest ones had a tendency to lose weight.

Another possible source of error, of which we did not become aware until the experiments were well under way, is the variation which some observers have detected in the vitamin C content of lemon juice. Bennett and Tarbert ('33), working in Sicily, using the dichlorophenolindophenol titration, found orange juice higher and more consistent than lemon juice in vitamin C content. This was confirmed by Harris and Ray ('33 b) in Great Britain, who found that orange juice consistently contained 0.6 mg. of ascorbic acid per cubic centimeter; the 'best' lemon juice equalled this, while inferior lemons yielded only 0.4 mg. of ascorbic acid per cubic centimeter. In our experiments, begun before the above cited papers were published, we decided on the use of decitrated lemon juice because it was the international standard. In the light of recent knowledge, ascorbic acid, rather than either orange or lemon juice, would be the ideal material to use in an investigation of this sort.

The sections of the incisor roots were prepared by the Department of Pathology, who have kindly furnished us with a description of the method which they developed. In brief, it consisted of a double embedding, first in celloidin, followed by decalcification with 5 per cent nitric acid, and then in paraffin. Sections of 7 μ were cut under 60 per cent alcohol on a celloidin microtome, and ordinary hematoxylin-eosin staining was employed. This method differs from the original one of Höjer ('26) and from the modifications described by Goettsch and Key ('28) and by Key and Elphick ('31) as well as that used in Prof. W. H. Eddy's laboratory,[3] but the slides revealed the changes as described by these investi-

[3] Personal communication.

gators. Diagnosis was made on the 0 to 4 scale of Key and Elphick, in which 4 indicates complete protection. The slides were evaluated without reference to the other data on the animals, and in all cases where there was any room for doubt, the decision represents the judgment of both authors.

RESULTS

A plot of all the data is presented in chart 1. The abscissae and ordinates represent vitamin C dosage and body weight, respectively; different results are indicated by appropriate symbols. The points at which complete protection from scurvy occurred—value 4 on the scale used—are represented by solid black circles, and are of greatest significance. The light circles indicate degree of protection 3, and the triangles 2 or less. For the purpose of the present investigation the distribution of the black circles, representative of complete protection, is of particular interest. The dotted line has been drawn to show how strongly these points indicate a definitely linear relationship between the weight of the animal and the dose of lemon juice necessary to protect it from scurvy as shown by examination of the teeth.

Tabulation of the limited number of cases which includes the heaviest animal protected by each dose (table 1) reveals that, with but two exceptions, the protective dose ranges from 0.72 to 1.22 cc. of lemon juice per 100 gm. of body weight; the average value for nine different dosages, varying from 1.5 to 9 cc., proves to be 0.97 cc. (approximately 1 cc.) per 100 gm. of body weight. This result is further supported by consideration of the number of borderline cases in which the dose was evidently just below the protective amount, resulting in a microscopic section of the incisor root which justified a diagnosis of '3.' The reason why we consider it valid to ignore the two exceptional cases is that, as may be seen from chart 1, in each instance where an unusually big guinea pig was protected by a given dose (1 cc. and 3.5 cc.) at least two smaller animals failed to be protected by this amount. The reason for the exceptions is not apparent.

In only two instances were the animals found to be pregnant. These cases are too few to suggest any conclusion, except that they present no evidence of an appreciable effect of pregnancy on the vitamin C requirement.

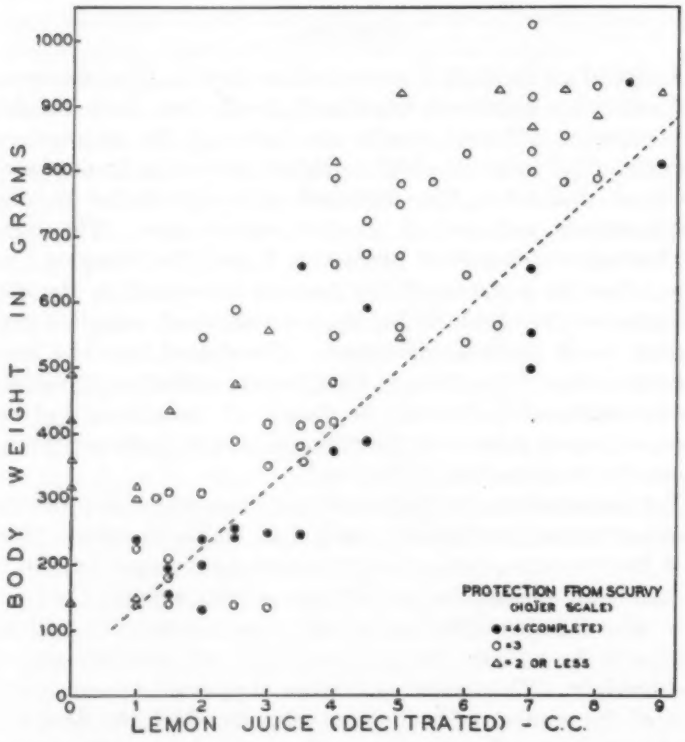

Chart 1 This plot of the body weights of guinea pigs against cubic centimeters per day of decitrated lemon juice (1932 International standard for vitamin C) indicates the degree of protection afforded against pathological changes in the odontoblastic cell layers of the roots of the lower incisor teeth. The black circles represent complete protection or a value of 4 on a scale of 1 to 4; the open circles indicate almost complete protection or a scale value of 3; in the cases represented by the triangles the protection afforded is almost nil, the scale values being 2 or less. It will be noted that the amount of vitamin C required to secure complete protection—black circles—is practically in linear relation to the body weight of the animals, and very nearly equal to 1 cc. of the decitrated lemon juice per 100 gm. of body weight.

With respect to the effect of age on the vitamin C requirement, the data suggest that the need of the youngest animals in proportion to body weight may be slightly less than that of the older groups; the observed difference is so small, however, that it may not be significant. At any rate, it is quite definitely apparent that there is no evidence of any added requirement because of the more rapid growth of the guinea pigs weighing less than 250 gm.

TABLE 1

Largest guinea pig protected by any given dose

DOSE	WEIGHT	DOSE
cc.	*gm.*	*cc. per 100 gm.*
1.0	227[1]	
1.5	209	0.72
2.0	237	0.84
2.5	252	0.99
3.0	245	1.22
3.5	652[1]	
4.0	373	1.07
4.5	589	0.76
7.0	648	1.08
8.5	935	0.91
9.0	805	1.11

[1] These cases are anomalous because at least two smaller pigs were not protected by the same dose.

In the animals studied in the winter of 1933–1934, the adrenals were stained with 0.4 per cent silver nitrate and evaluated on a purely qualitative basis. This was done in four groups of guinea pigs, containing from four to nine individuals in each group; as each group was sacrificed, the adrenals of all the members were stained for exactly 15 minutes each, and arranged in the order of intensity of the dark color. Considerable variation was observed. After the tooth slides were ready, this order was compared with the diagnosis and also with the dosage. It happened that in each group there was only one animal which was rated '4' on the Key and Elphick scale, and in every instance this turned out to be the one whose

adrenals had stained most deeply, and also to be the one which had received the largest dose of lemon juice in proportion to its body weight. This result is in accord with what might have been expected from the work of Moore and Ray ('32). However, in the cases of the animals not completely protected, little or no correlation between the intensity of staining and the degree of protection could be discovered. This observation substantiates the later work from the same laboratory (Harris and Ray, '33 a) as well as the earlier statement of Gough and Zilva ('33), that the failure of adrenal tissue to stain deeply with silver nitrate cannot be relied on as a test for the absence of vitamin C.

DISCUSSION

The data here reported seem to warrant the statement that the amount of vitamin C needed to protect a guinea pig from changes in the microscopic structure of the incisor roots is proportional to its body weight over a range of 120 to 1000 gm. To assert further that the vitamin C requirement of the whole body is proportional to body weight in this species obviously involves the assumption that the tooth changes furnish a reliable index of early vitamin C deficiency of the entire organism. The validity of this assumption seems to be unquestioned by those who have used this method. Nevertheless, it might be argued that the odontoblasts, the dentine-forming cells which respond most conspicuously to diminished supply of vitamin C, might have a requirement for this dietary factor different from that of cells elsewhere in the body, because they are concerned in the growth of a structure which progresses throughout life. Dalldorf and Zall ('30) have shown that the normal rate of growth of a guinea pig's incisors is continuous, and is only slightly less in animals of 110 gm. body weight than in those weighing 600 gm. However, since the rate is practically independent of body size, one would expect that if the odontoblasts have any peculiar vitamin C requirement, this would also be independent of body size. The very fact that we have shown the requirement

for normal tooth structure to vary directly with body size seems conclusive evidence that deviation from the normal in this respect is indeed an index of the entire body's deficiency.

If, then, we are justified in asserting that the direct ratio between dosage and body size is valid, the relationship can be expressed by the simple formula:

$$\text{VITAMIN}_{\text{per day}} = \text{K.WEIGHT}$$

Because of the units used to express the two variables in the present work, namely, cubic centimeters of decitrated lemon juice and 100 gm. body weight, the value of K happens to be unity. It is obvious that the value would be much less if ascorbic acid were used for the assay instead of lemon juice. Harris and Ray ('33 b) state that, with respect to ascorbic acid, "the minimum dose for full tooth protection (under the conditions of the test) is 2 mg." In discussing the variable potency of lemon juice, these authors also report that 1 cc. of 'good' lemon juice is equivalent to 0.6 to 0.7 mg. of ascorbic acid. Using these data, it appears from our findings that the guinea pig requires about 0.6 to 0.7 mg. of ascorbic acid per 100 gm. body weight per day. For animals of the size commonly used in vitamin C assay, namely, 250 to 350 gm. weight, the daily minimum of ascorbic acid would be from 1.5 to 2.1 mg. The higher figure, taken as the safer minimum to use, agrees very well with the minimum as stated by Harris and Ray, namely, 2 mg. Our results expressed in the 1932 International Vitamin C units based on decitrated lemon juice, indicate that the guinea pig requires 10 International units per 100 gm. body weight per day; based on the 1934 International Vitamin C unit of 0.05 mg. ascorbic acid,[4] the requirement would be 12 to 14 units per 100 gm. body weight.

The relationship between the requirement for vitamin C and body weight in the one species studied is much simpler than that found by Cowgill and co-workers ('32) for vitamin B, expressed by the formula:

$$\text{VITAMIN}_{\text{per day}} = \text{K}_s\text{.WEIGHT}^{\frac{1}{3}}$$

[4] Personal communication from Dr. E. M. Nelson, of the Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

in which the value of K_s is peculiar to the species. This expression was found valid for pigeons, rats and dogs. The term $\text{WEIGHT}^{\frac{5}{3}}$ can be considered as equivalent to $\text{WEIGHT} \times \text{WEIGHT}^{\frac{2}{3}}$, and in interpreting this with respect to the vitamin B requirement of several different species, Cowgill concluded that "the vitamin requirement per unit of mass, i.e., weight, is proportional to the metabolism of that mass, that is, the two-thirds power of the weight."

The difference between the two formulas cited above merits some discussion. In the case of the expression pertaining to vitamin B, where the five-thirds power is the function of the body weight of interest, the formula was interpreted to indicate that not only mass (three-thirds power of the weight), but rate of metabolism (two-thirds power of the weight) as well, is an important determinant of the vitamin requirement. The absence of this latter factor from the vitamin C formula suggests that rate of metabolism does not play a role in determining the need for ascorbic acid. Against this view one may mention the fact that vitamin C is not merely an inactive component of certain tissues, but must be involved in metabolic transformations resulting in its destruction or inactivation because it is necessary to supply the vitamin frequently in order to obviate the onset of deficiency symptoms. If one wishes to adopt the suggestion of Wolbach and Howe ('26) and think of each vitamin as playing some important role in the functioning of a particular tissue, one would doubtless relate vitamin C to connective tissue, which has a relatively low rate of energy exchange in comparison with other tissues of the body; and on this latter fact one might base an explanation of the finding that rate of metabolism seems to play no role in determining the vitamin C requirement. In all probability the rate of metabolism exerts some influence on the need for vitamin C, but one which is extremely small in comparison with its effect on the vitamin B requirement, and therefore not appearing as a significant variable in the derived formula. It would be of interest to determine the effect of administration of thyroid tissue or of thyroxin on the re-

quirement for vitamin C. The results of such studies should contribute much in relation to this question.

Our findings with reference to the guinea pig seem to be at variance with those of Falk, Gedda and Göthlin ('32–'33), who report that the child needs two to three times as much vitamin C in proportion to body weight as the adult human being. In this connection it is pertinent to point out, first, that their criterion of vitamin C deficiency consists in testing the strength of skin capillaries, and is therefore very different from ours, which is based on a study of the condition of the roots of the incisor teeth; and, second, that the two sets of observations were made on different species. It is possible that a species difference exists, especially in view of the fact that growth in the human being, up to the age of puberty, is enormously slower than in the guinea pig and other animals. This difference is beautifully illustrated by the growth charts of Brody ('27). We might also point out that the conditions of our experiments were not framed to deal critically with the growth factor. Until experiments on guinea pigs especially aimed at elucidation of the effect of growth per se on the vitamin C requirement have been performed, we do not feel justified in drawing conclusions with respect to this point.

We wish to thank Dr. Marianne Goettsch for suggestions relating to the preparation of the microscopic sections of the incisor roots and for lending us a set of standard slides. We also wish to express our appreciation of personal communications received in response to our inquiries from Dr. Kathleen M. Key, and from Dr. Celia Gurin, of Dr. W. H. Eddy's laboratory, which were helpful in formulating our plan of procedure in this investigation.

SUMMARY AND CONCLUSIONS

The vitamin C requirement was determined for eighty guinea pigs weighing between 120 and 1040 gm. Each animal, while subsisting for 2 weeks on a scorbutic diet, was given a daily dose of decitrated lemon juice varying, for different

individuals, from 0 to 2.2 cc. per 100 gm. of body weight, but mainly in the range of 0.5 to 1.5 cc. per 100 gm. The animals were chloroformed and the degree of protection from scurvy, as indicated by the microscopic structure of the roots of the incisor teeth, was evaluated by Key and Elphick's scale, adapted from the Höjer method.

The results indicate that the vitamin C requirement of the guinea pig is directly proportional to the body weight, and is almost exactly 1 cc. of lemon juice per 100 gm. This confirms the observations of Goettsch and Key, Eddy and other investigators, for guinea pigs of about 300 gm. of body weight, and applies to a much wider range of body size.

There is no evidence from these data that the young, rapidly growing guinea pig requires a proportionately greater amount of this dietary factor than the adult. This conclusion differs from that of Göthlin and his co-workers with respect to the human requirement, and may be due to a species difference.

Silver nitrate staining of the adrenals was found to be of only slight value as a criterion of the degree of protection from scurvy, as compared to the more accurate technic based on the changes in the roots of the incisors.

The role of the metabolic rate, which in the case of vitamin B has been found to be of equal importance to body weight as a determinant of the requirement of various species for the vitamin, appears insignificantly small so far as vitamin C is concerned.

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THE EFFECTS OF LACTOSE ON GROWTH AND LONGEVITY

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FIVE FIGURES

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INTRODUCTION

In spite of the tendency in times past for pediatricians to emphasize the advantages of the high degree of assimilability of certain carbohydrates, such as sucrose, maltose and glucose, and to discourage the use of lactose in infant feeding, it has, more recently, been generally recognized that lactose has several uniquely characteristic properties that, at least for certain nutritional purposes, are of definite value. The authority on which lactose was to some extent discarded in favor of other sugars was derived from very meager clinical evidence and from unjustified interpretations of results of evidently sound experimentation. Even the pertinence of the fact that the solids of human milk contain 50 per cent lactose seems to have been frequently disregarded in considerations of synthetic diets for infants. This whole question has been discussed by Jarvis ('30), who was one of several to reinvestigate the effects of lactose in infant feeding.

Jarvis ('30) concluded from work with infants and from related experiments with rats that "age-weight for age-weight, the lactose-fed infant possesses more living tissue than does the infant fed on vegetable sugar." Barenberg and Abramson ('30) carried out similar work, using as much as

15 per cent of lactose in the ration, and found that infants receiving milk sugar responded with a better rate of growth than did the control group. Ariyama and Takahasi ('29) found that, in spite of the comparatively small coefficient of absorption of lactose observed by them, it had more effect on the growth of rats than sugars having greater absorption coefficients, especially in the early part of the feeding period. Mitchell ('27), feeding rats on rations containing 60 per cent of each of five common carbohydrates, respectively, found growth to be the poorest on lactose on account of lower digestibility and resulting diarrhea. Bergeim ('26) and several other investigators (Gross, '27; Robinson et al., '29), more recently, have shown that lactose feeding causes considerable increases in the amounts of calcium and phosphorus absorbed.

With the purpose of verifying some of these results on the effect of lactose feeding on the growth of young animals and of determining whether lactose has any significant effect on the body weights of adult animals and on longevity, feeding experiments with rats and pigs were carried out at the Beltsville, Md., laboratories of these bureaus.[1] Comparisons were made of body composition of animals fed lactose with that of animals fed other carbohydrates.

DESCRIPTION OF EXPERIMENTS

Three groups of rats were used in this work. Group I consisted of fourteen pairs used for the comparison of the effects of lactose and sucrose on growth and longevity and nine pairs used for the comparison of the effects of lactose and dextrin. Each pair consisted of litter mates of the same sex, but different ages. Up to the time of being placed on the experiment, the rats had received the stock ration of the breeding colony. From then on they were fed ad libitum either the

[1] S. S. Buckley (since deceased) and Paul E. Howe, Bureau of Animal Industry, planned the details of the work with pigs. Charles Hufnagel, Bureau of Dairy Industry, was in charge of feeding the rats. P. N. Peter (since deceased), S. P. Gould and L. A. Shinn, Bureau of Dairy Industry, did the analyses on the rats.

lactose ration, the sucrose ration, or the dextrin ration, as listed in table 1. The lactose used was a commercial product, containing, presumably, all the vitamins usually present in this grade of lactose. Figure 1 shows the average changes in weight with age of each sex group on lactose and on sucrose. Figure 2 shows similar data up to 180 days of age for the rats paired on lactose and dextrin.

Several pairs of rats on the sucrose-lactose comparison were killed at 132 to 159 days of age and were analyzed. Results

TABLE 1

Composition of the rations fed to rats in the different groups

RATION	COMPOSITION									FED TO RATS IN GROUPS
	Casein[1]	Salt mixture[2]	Cod-liver oil	Agar	Yeast[3]	Lard	Dextrin	Lactose[1]	Sucrose[1]	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	
I (lactose)	18	4	2.5	2	5	5	33.5	30.0	00.0	I and II
II (sucrose)	18	4	2.5	2	5	5	33.5	00.0	30.0	I and II
III (dextrin)	18	4	2.5	2	5	5	63.5	00.0	00.0	I
IV (45% lactose)	18	4	2.5	2	5	5	18.5	45.0	00.0	III
V (45% sucrose)	18	4	2.5	2	5	5	18.5	00.0	45.0	III
VI (63.5% lactose)	18	4	2.5	2	5	5	00.0	63.5	00.0	III
VII (63.5% sucrose)	18	4	2.5	2	5	5	00.0	00.0	63.5	III

[1] Commercial.
[2] Osborne and Mendel.
[3] Northwestern Yeast Company (Chicago), 'Yeast Foam Tablets Powder.'

of these analyses are given in table 2. Nine pairs were continued on the sucrose and lactose rations to obtain data on the relative effects of these sugars on the span of life. Longevity data are given in table 3. In only a few cases was it possible to determine by post-mortem examination a definite pathological cause of death. Pneumonic indications, lung abscess, enlarged spleen, tumor, and paralysis were observed, but none of these was consistently the immediate cause of death.

Group II consisted of ten pairs, each pair being litter mates of the same age. This group was started on the experiment

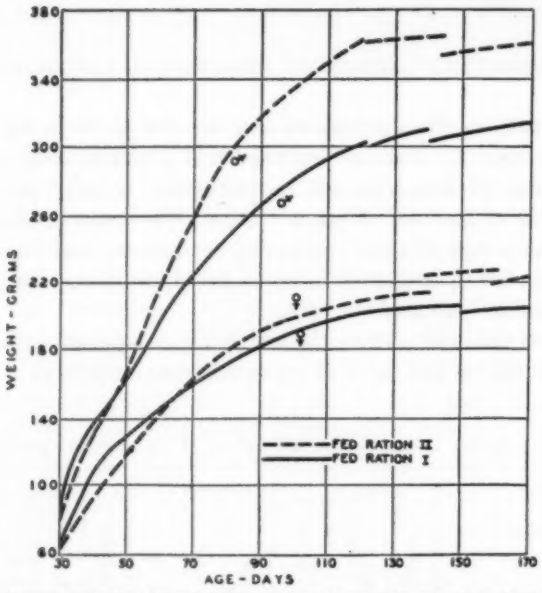

Fig. 1 Growth of rats fed ad libitum on rations I (lactose) and II (sucrose). Breaks in the curves occur, because some of the rats were killed for analysis during the feeding period.

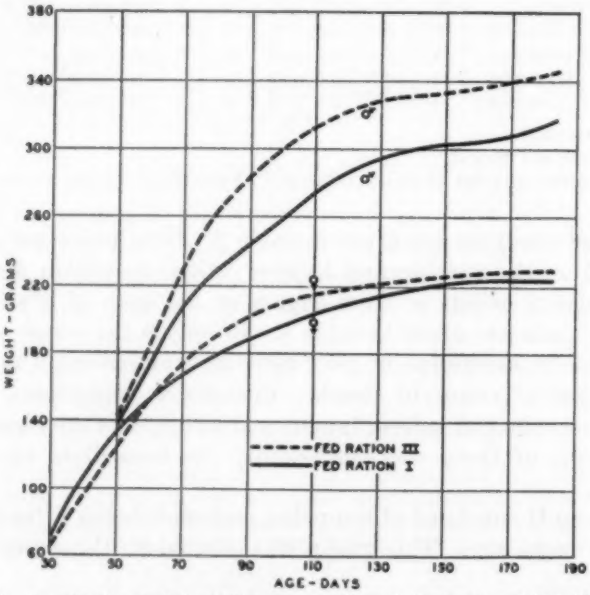

Fig. 2 Growth of rats fed ad libitum on rations I (lactose) and III (dextrin).

TABLE 2

Analyses of four pairs of rats in group I, killed at 132 to 159 days of age

	MALES						FEMALES					
	Pair 1[1]			Pair 6[1]			Pair 7[1]			Pair 11[1]		
	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Live weight	326.0	388.0		380.0	417.0		249.0	250.0		223.0	199.0	
Intestinal fill	19.0	12.6		13.4	11.8		17.3	12.9		12.4	10.8	
Net body weight	307.0	375.4	68.4	366.6	405.2	38.6	231.7	237.1	5.4	210.6	188.2	– 22.4
Moisture in net body weight	171.7	182.7		214.5	218.2		127.4	122.9		131.7	102.6	
Net dry body weight	135.3	192.7	57.4	152.1	187.0	34.9	104.3	114.2	9.9	78.9	85.6	6.7
Fat in net body weight	62.2	115.0	52.8	65.7	103.6	37.9	49.1	64.9	15.8	25.3	45.3	20.0
Protein in net body weight	60.2	64.7		74.7	73.7		42.3	40.9		44.7	33.5	
Ash in net body weight	10.5	11.2		9.5	8.4		8.6	7.7		7.7	5.5	

[1] Age when killed: pair 1, 132 days; pair 6, 150 days; pair 7, 159 days; and pair 11, 146 days.

TABLE 3

Span of life of rats on lactose and sucrose rations

GROUP	PAIR	AGE OF LACTOSE-FED RAT AT DEATH	AGE OF SUCROSE-FED RAT AT DEATH	DIFFERENCE
		days	days	days
I	2	897	336	561
I	3	951	386	565
I	4	867	573	294
I	5	879	1095	– 216
I	9	877	499	378
I	10	720	627	93
I	15	1040	814	226
I	16	986	1070	– 84
I	17	908	687	221
II	1	785	790	– 5
II	2	1000	780	220
II	3	899	676	223
II	4	992[1]	779	213
II	6	340	340[2]	
II	7	555[2]	555	
II	8	566	977[1]	– 411
Average group I		903	676	227
Average group II		848	800	48
Average of all		883	720	163

[1] Still alive when this table was made. They are included in averages.

[2] Died before its mate. Mate was then discarded. Neither is included in averages.

a year later than group I. Sucrose and lactose rations of the same composition as before were used, but each rat was kept in a separate cage and members of each pair, being allowed as much food as the less avid member would eat, consumed practically the same quantity. The rats of this group were started on the experiment at weaning. Weight changes are shown in figure 3, and data on life span for this group are in

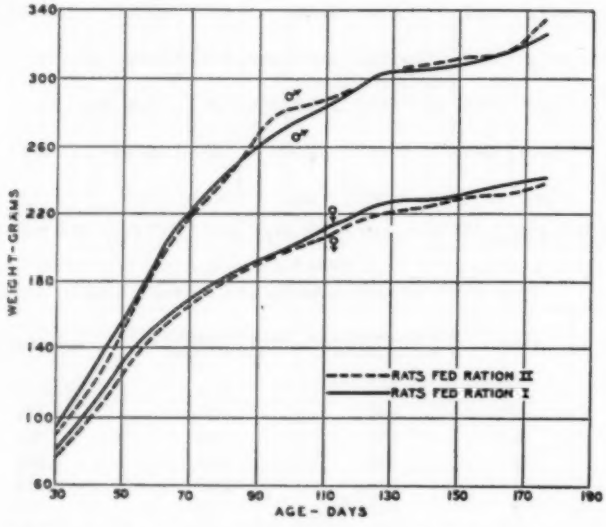

Fig. 3 Growth of rats on rations I (lactose) and II (sucrose), members of each pair were fed the same quantity.

table 3. Post-mortem examinations revealed nothing significant. Two pairs of rats in group II were killed and analyzed, the results being given in table 4.

Group III, consisting of twelve rats, was used to determine the effects of larger proportions of sucrose and of lactose in the rations—45 and 63.5 per cent. The results are shown in figures 4 and 5, the rations used being listed in table 1. The rats tolerated the large amounts of sucrose very well, but all of the rats on the high lactose rations developed diarrhea

and did not gain weight satisfactorily for about 3 weeks. At the end of that time, however, they recovered from the diarrhea and began good gains, in spite of the fact that they were consuming the large amounts of lactose. In a short time, the

TABLE 4

Analyses of two pairs of rats in group II, killed at 774 days of age

	FEMALE PAIR			MALE PAIR		
	Lactose-fed	Sucrose-fed	Difference	Lactose-fed	Sucrose-fed	Difference
	gm.	gm.	gm.	gm.	gm.	gm.
Live weight	280.7	269.0	....	345.3	360.9	....
Intestinal fill	7.4	9.0	....	19.8	11.5	....
Net body weight	273.3	260.0	−13.3	325.5	349.4	23.9
Moisture in net body weight	153.2	141.0	....	180.6	193.7	....
Net dry body weight	120.1	119.0	−1.1	144.9	155.7	10.8
Fat in net body weight	59.7	62.5	....	71.1	80.5	....
Protein in net body weight	48.0	45.6	−2.4	58.5	62.0	3.5
Ash in net body weight	10.5	9.3	....	12.1	12.0	....

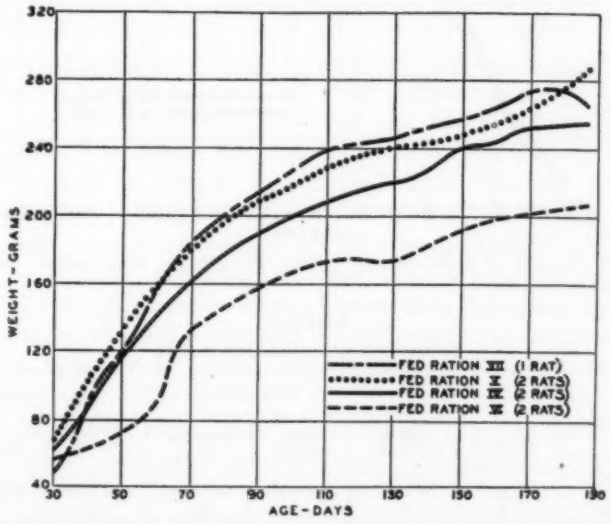

Fig. 4 Growth of female rats on rations IV (45 per cent lactose), V (45 per cent sucrose), VI (63.5 per cent lactose), and VII (63.5 per cent sucrose).

rats receiving the 45 per cent lactose ration attained practically the same weights as their partners on the same percentage of sucrose. The rats receiving the 63.5 per cent lactose ration were slower in overtaking their mates.

In the experiments with pigs, the rations listed in table 5 were used. The salt mixture contained 50.00 per cent lime-

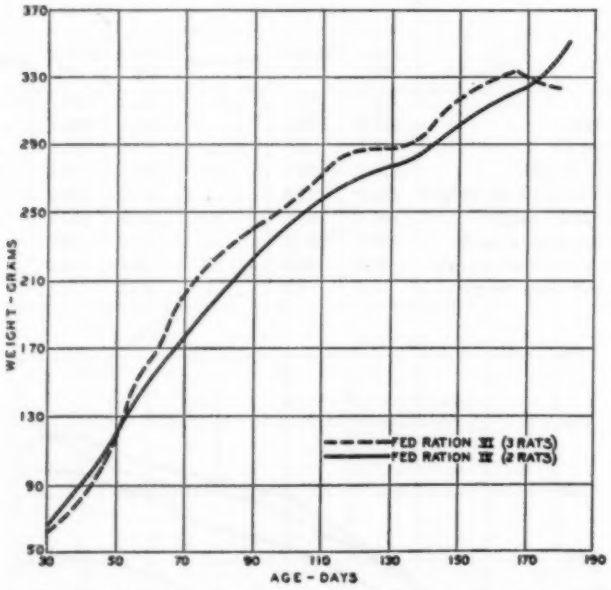

Fig. 5 Growth of male rats on rations IV (45 per cent lactose) and VI (63.5 per cent lactose).

TABLE 5

Composition of rations fed to pigs

RATION	COMPOSITION					
	Tankage	Alfalfa leaf meal	Salt mixture	Brewers' rice	Sucrose	Lactose
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Check	9	5	1	85	0	0
Sucrose	14	5	1	40	40	0
Lactose	14	5	1	40	0	40

stone, 27.97 per cent steamed bone meal, 20.00 per cent NaCl, 2.00 per cent Fe_2O_3, 0.01 per cent $CuSO_4$, and 0.02 per cent KI.

Twelve pigs were fed in four groups of three each, each pig of a group having a different ration and being fed separately. The pigs were allowed as much of the ration as the least avid member of each triplet would consume. The first week, the sugar rations contained 10 per cent sugar and 70 per cent brewers' rice; the second and third weeks, 20 per cent sugar and 60 per cent brewers' rice was fed; thereafter, the rations were fed as listed in the table. The total feeding period was 25 weeks, from February 9, 1932, to August 8, 1932. The pigs were weighed at the beginning of the experimental feeding; at the end they were weighed, slaughtered, and the edible portions of the carcasses analyzed. The results are tabulated in table 6.

DISCUSSION

During the period when the rats of groups I and II were very young, those that were fed lactose increased in weight much faster than their mates on the sucrose ration. After attaining what may be called adult age, the sucrose-fed rats of group I became heavier than their lactose-fed partners and remained considerably heavier on the average for the remainder of their lives. In group II there was a slight tendency for the adult sucrose-fed rats to be heavier than the lactose-fed rats, but the differences on the controlled feeding basis were less than those on the ad libitum feeding of group I. This is readily explainable, since the sucrose-fed rats of group I evidently consumed much more feed than their lactose-fed mates, probably because of difference in palatability, lactose being only about one-sixth as sweet as sucrose (Biester et al., '25).

Dextrin parallels sucrose rather than lactose in its effects, when fed in moderate amounts, thus indicating that supplying carbohydrate to the lower intestine for stimulation of acidophilic organisms is probably not the factor causing the differentiation in the effects of lactose and sucrose.

TABLE 6

Growth and composition of pigs fed sucrose and lactose rations

RATION FED	GROUP	LITTER AND INDIVIDUAL NUMBER[1]	BREED[2]	SEX	WEIGHTS		GAIN		FEED CONSUMED PER POUND GAIN	COLD DRESSED CARCASS	TOTAL EDIBLE MEAT	CHEMICAL COMPARISON OF TOTAL EDIBLE				FIRMNESS	REF. INDEX 40°
					Initial	Final	Total	A.D.G.				Water	Ash	Fat	Protein		
					lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*per cent*	*per cent*	*per cent*	*per cent*		
Control	A	304.2	DJ × Tam.	M	67	179	112	0.63	4.28	131.5	96.02	42.91	0.62	44.32	11.92	H	1.4589
	B	6.4	C.W.	F	71	189	118	0.73	5.18	146.5	108.46	40.11	0.52	48.53	11.10	MH	1.4591
	C	213.1	P.C.	F	88	186	98	0.55	5.41	147.5	105.34	48.78	0.66	37.55	13.29	MS	1.4594
	D	8.3	C.W.	F	82	212	130	0.81	4.74	168.5	122.86	46.69	0.54	39.08	13.51	H	1.4587
Average					77	191.5	114.5	0.68	4.90	148.5	108.42	44.62	0.58	42.37	12.46		1.4590
Sucrose	A	8.5	C.W.	F	62	127	65	0.37	7.15	97.5	69.22	41.32	0.58	46.95	10.28	S	1.4597
	B	6.3	C.W.	M	80	165	85	0.63	6.01	Died near the end of the experiment							
	C	213.5	P.C.	F	85	190	105	0.59	5.05	149.0	108.62	41.36	0.53	47.78	10.64	MS	1.4595
	D	304.5	DJ × Tam.	M	89	181	92	0.57	6.72	135.5	94.76	38.73	0.40	50.68	10.38	MS	1.4595
Average for 3					78.6	166	87.3	0.54	6.23	127.3	90.87	40.47	0.50	48.58	10.43		1.4596
Lactose	A	8.7	C.W.	F	59	139	80	0.45	5.79	110.0	76.12	48.95	0.63	38.41	11.90	MH	1.4589
	B	6.2	C.W.	M	88	189	101	0.63	6.03	144.0	101.32	47.49	0.57	39.03	13.54	MH	1.4594
	C	213.6	P.C.	F	85	148	63	0.36	8.57	113.0	80.08	47.93	0.67	39.66	12.34	MS	1.4594
	D	103.9	D.J.	M	84	185	101	0.63	6.13	137.0	94.62	43.56	0.68	43.94	12.05	MH	1.4589
Average					79	165.2	86.2	0.52	6.63	126.0	88.04	46.98	0.64	40.26	12.46		1.4592

[1] That is, 304.2 signifies pig no. 2 of litter no. 304.

[2] DJ × Tam. = Duroc-Jersey Tamworth cross; C.W. = Chester White; P.C. = Poland China.

The data of table 2 show clearly that the differences in weight of adult rats fed ad libitum on the lactose and the sucrose rations are accounted for practically entirely by differences in the amounts of fat present in their bodies. Definitely more fat is present in the bodies of sucrose-fed females in this group even when their body weights are no greater than those of their lactose-fed partners. These results have been confirmed by the previously mentioned work with pigs. Of interest in this connection is an observation of Kotchneff ('24) that lactose is almost entirely retained by the liver as glycogen.

As shown by table 3, the sucrose-fed rats of group I died sooner than their mates in seven comparisons out of nine. The same tendency was manifest in group II. We do not suggest any explanation of these observations on longevity.

The results of feeding the 45 and the 63.5 per cent sugar rations point to the reason for Mitchell's findings ('27) that growth on a lactose ration is comparatively poor. After the period of adaptation, during which the diarrheal condition and slow rate of growth were the most evident features of the lactose feeding, our rats not only grew satisfactorily but practically overtook the sucrose-fed rats, which had not been retarded by intestinal disturbances.

Results of the experiments with pigs show that neither the sucrose nor the lactose ration was as suitable for pigs as was the control. The fat percentages of the carcasses of the hogs fed the sucrose ration were considerably greater than those of the other two lots, this fact substantiating the results on rats in this particular. The firmness grades and the values of refractive index of the fat show that the flesh of the sucrose-fed hogs was softer than that of the hogs on either of the other rations.

SUMMARY

Lactose caused more rapid growth of young rats than did sucrose.

Adult rats became heavier on a sucrose ration than on a lactose ration when fed ad libitum, a difference attributed to greater accumulation of fat in the bodies of the sucrose-fed rats.

Pigs fed sucrose accumulated a greater proportion of fat than did pigs fed lactose.

The characteristic results obtained on rate of growth on lactose rations are evidently not to be explained on the basis of stimulation of acidophilic organisms in the lower intestine, since dextrin has not the same influence on growth as has lactose.

Lactose-fed rats, in general, lived longer than sucrose-fed rats.

Feeding of excessive quantities of lactose to rats caused diarrheal conditions and retardation of growth for a short time, after which the disadvantage was practically overcome.

It is suggested that these results may be directly applicable to human nutrition.

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